CHAPTER I

GENERAL MORPHOLOGY OF THE CELL

"We have seen that all organisms are composed of essentially like parts, namely, of cells; that these cells are formed and grow in accordance with essentially the same laws; hence, that these processes must everywhere result from the operation of the same forces."

SCHWANN.[1]

Schwann first gave clear expression to the fundamental conception that beneath unending diversity of form and function all cells conform to a common morphological and physiological type. Like Schleiden, it is true, he failed to grasp the real nature of the cell, considering the protoplasmic cell-contents as of minor importance; nevertheless the essential truth of his sweeping generalization was established by later investigation on an ever widening basis. Even to-day we cannot frame an adequate brief definition of the cell; but fortunately such a definition is unnecessary. In practice we need no more than the simple formula put forward long ago by Leydig and Max Schultze, and still in everyday use. The cell, according to this definition, *is a mass of protoplasm* (in modern terminology the *cytosome*) *containing a nucleus;* and to this may be added Schultze's statement that *both nucleus and cytosome*, arise *by division of the corresponding elements of a preëxisting cell.*[2] This definition must not be taken in too formal or narrow a sense. Like most other definitions in natural science it must be allowed a certain flexibility, but in respect to essential accuracy the old definition remains to-day unshaken by the advances of half a century.

The general sketch of the cell here offered is but a bare outline. Many of the topics touched upon will be more critically discussed in later chapters.

I. GENERAL SKETCH. INTRODUCTORY

The early writers applied the term "protoplasm" [3] to the substance of the cell-body or cytosome in contradistinction to that of the nucleus; and the word (often shortened to *plasma*) is still commonly used in the same sense by modern writers. Later it acquired a broader significance, often

[1] *Untersuchungen*, 1839, p. 227.

[2] Leydig, *Lehrbuch der Histologie*, 1857, p. 9; Schultze, *Arch. Anat. u Phys.* 1861, p. 11.

[3] This word, nearly equivalent, etymologically and in meaning, to the "Urschleim" of Oken (1801), was first employed by Purkinje (1840) to designate the formative material of the animal embryo, and later applied by Mohl to the contents of plant cells. Beale (1870) proposed the appropriate word *bioplasm* as a substitute for protoplasm, but this has never come into general use.

being applied to the cell-substance as a whole, including the nucleus. In the interest of greater precision, therefore, Strasburger ('82) proposed to designate the substance of the cytosome as *cytoplasm* and that of the nucleus as *nucleoplasm* (better *karyoplasm*), both being included under the more general term "protoplasm." This terminology has been widely adopted, and we shall continue to use it; nevertheless, when we speak of "protoplasm" we commonly have in mind the earlier use of the word, *i. e.*, as equivalent to "cytoplasm."

Cytosome and nucleus taken together form a living unit or protoplasmic system that is often spoken of as the *protoplast* (Hanstein) or sometimes as the *energid* (Sachs). Externally the cytosome is bounded by a thin, peripheral, clear, protoplasmic film of different consistency, the *plasma-membrane* (sometimes called the *ectoplast*), and it may also be surrounded by non-protoplasmic walls or "true membranes" of varied nature which in the tissues form partitions between contiguous cells. In both plants and animals, however, the cell-walls are often traversed by fine strands of protoplasm (plasma-bridges, or plasmodesms) by means of which a direct protoplasmic continuity is maintained between the protoplasts. In plants, as a rule, the cell-walls are harder, thicker and more conspicuous than in animals, and to this circumstance the unlucky term "cell" owes its origin. For the walls of such tissues, when viewed in section, give an appearance like that of a honeycomb often in the older tissues emphasized by death and disappearance of the protoplast so as to leave only the lifeless walls; hence the term "cell," first employed by botanists of the seventeenth century.[1] Here, too, was the source of the erroneous view of Schleiden and Schwann that the cell-wall is the most important part of the cell. The living protoplasm of the cytosome was at first overlooked or regarded as a waste-product. The researches of Dujardin, De Bary, Cohn, Max Schultze and many others long since showed, however, that most living cells are not hollow but solid bodies, and that in many cases—for example, the cells of blood and lymph or various *Protista*,—they are naked masses of protoplasm. Thus it was proved that neither the vesicular form nor the presence of a surrounding wall is an essential character of the cell and that the *cell-contents*, *i. e.*, the cytosome and nucleus, must be the seat of vital activity. The term "cell" thus became a biological misnomer. In the older cells of plants, it is true, the cytosome itself often becomes sac-like through the appearance of watery vacuoles which enlarge and finally fuse to form a single large central vacuole, surrounded by a thin peripheral

[1] As first employed by Robert Hooke (1665) the word was used to designate the minute cavities separated by solid walls, observed in cork, a tissue which he described as made up of "little boxes or cells distinct from one another."

layer (the "primordial utricle" of earlier botanists), though often traversed also by anastomosing strands of protoplasm. In such cases the living protoplasm does indeed assume the form of a hollow chamber; but this is

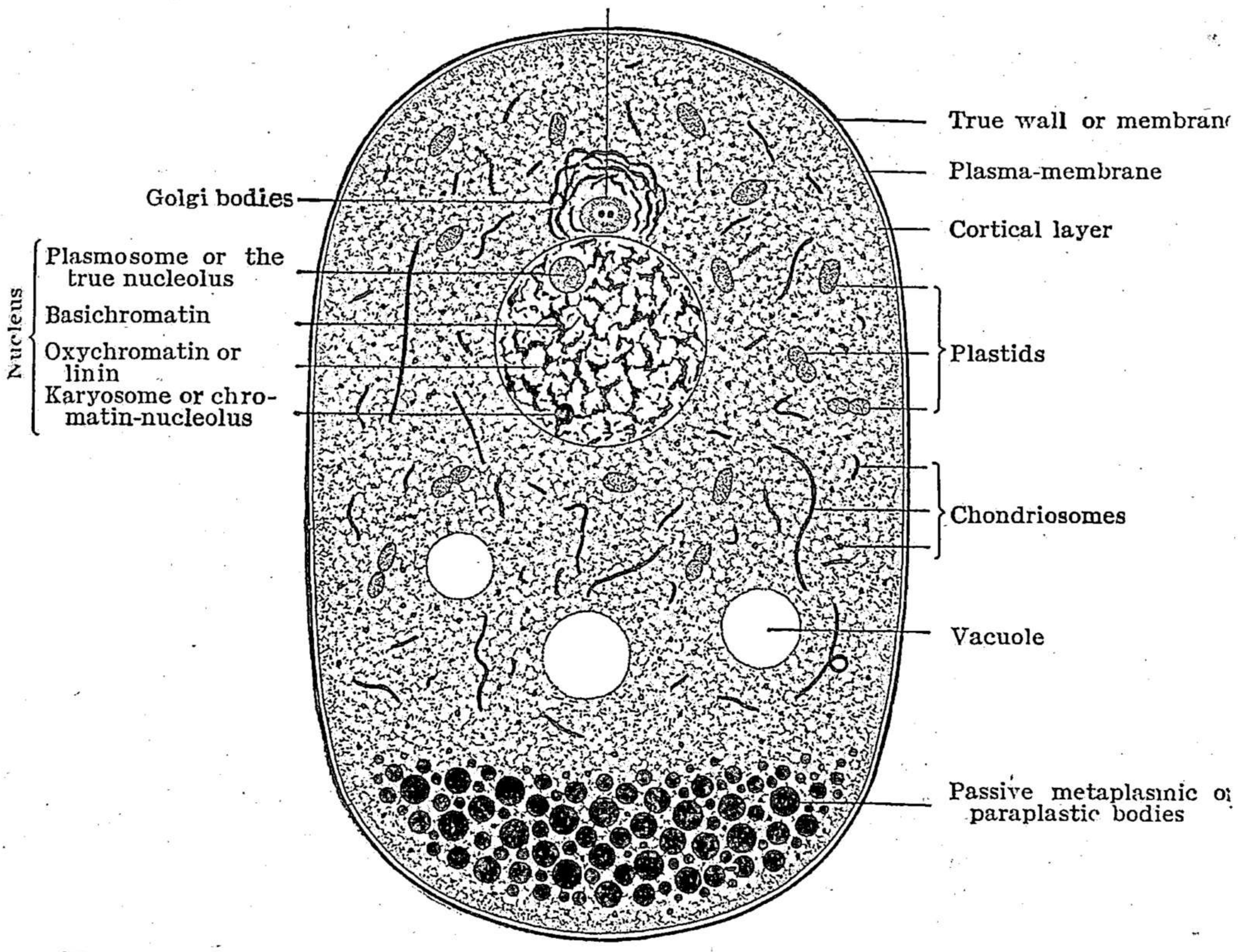


Fig. 6.—General diagram of a cell. Its cytoplasmic basis is shown as a granular meshwork or framework in which are suspended various differentiatied granules, fibrillæ and other formed components.

of secondary origin and significance. In their young and less differentiated condition these same cells are solid, like those of animals generally.

The nucleus (Figs. 6 and 8)[1] is typically of definite, rounded form, and often contains one or more smaller *nucleoli*. In all ordinary cases the nucleus is single, but in some cells two or more nuclei are present. Examples of cells that are constantly binucleate are offered by the sporophytic generation of the rusts and certain other fungi (Fig. 309), by the ciliate Infusoria generally, and by certain of the rhizopods (*e. g.*, *Arcella*, *Amœba diploidea*) (Fig. 296), flagellates (*Giardia*, Fig. 43) and Sporozoa. In the so-called *polymorphic* nuclei, *e. g.*, in some forms of leucocytes or in the giant-cells

[1] The nucleus was seen by Fontana in 1781, but was emphasized as a characteristic element of the cell by Meyen (1826), and especially by Robert Brown in 1831.

of the bone-marrow (Figs. 10 and 34) the nucleus consists of a nest or group of more or less separate vesicles or sacculations. In other cases several or many separate nuclei lie scattered in a common protoplasmic mass; such a structure is known as a *syncytium*, or (in the case of Protista) a *plasmodium*. This condition frequently occurs among the Radiolaria and other rhizopods and is characteristic of the so-called "non-cellular" fungi

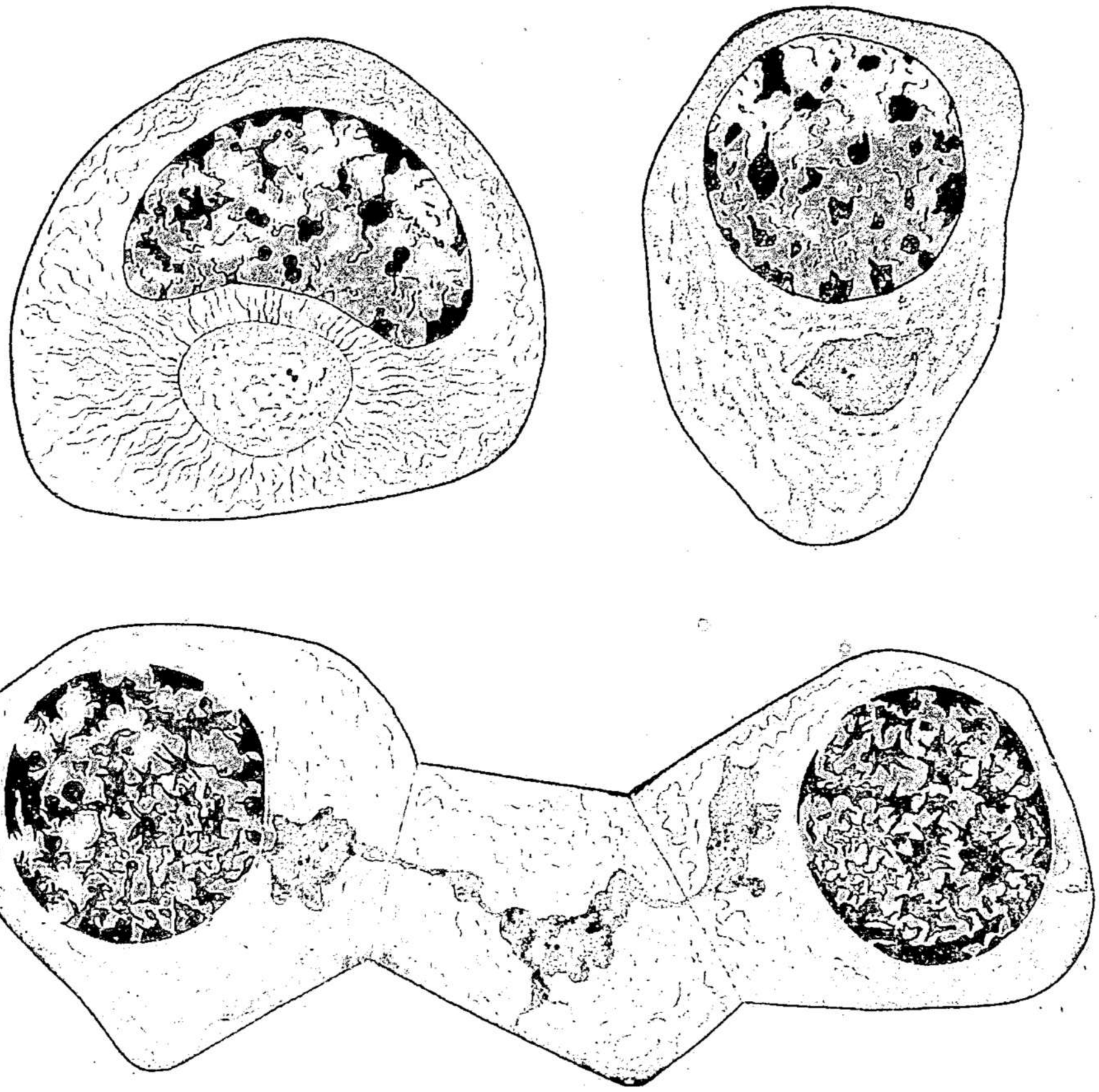


Fig. 7.—Spermatogonia of the salamander (MEVES). Above, two cells showing large nuclei, with linin-threads and scattered chromatin-granules; in each cell a centrosome or idiozome with two centrioles. Below, three contiguous spermatogonia, showing chromatin-reticulum, centriole and spindle-remnants.

such as *Mucor* and other Phycomycetes, and algæ such as *Caulerpa* or *Vaucheria* (cœnocytes).

The nucleus was long supposed to be absent in some of the Protista; and for such forms, Haeckel set up a group of so-called *Monera*, in which the body was supposed to be no more than a minute and homogeneous mass of protoplasm. Later a similar view was held in regard to the Bacteria and Cyanophyceæ. With the improvements of cytological technique and the general advance of protistology this conception was progressively re-

stricted and at last abandoned by nearly all investigators. Some of the "Monera" were found to possess single nuclei of the ordinary type; others to be multinucleate, with many small nuclei; still others to contain numerous minute *chromidia* in the form of granules, clumps or net-like formations scattered through the protoplasm and forming a diffuse or "distributed" nucleus, or nuclear system. Such chromidial formations appear to be not uncommon in lower plants and animals including certain Bacteria, Cyanophyceæ, rhizopods, flagellates and even ciliates (Figs. 14, 32). Identification of the chromidial substance as of nuclear nature or as "chromatin" rests in part on its staining-reactions and resistance to peptic digestion (p. 643); and the chromidial granules have been asserted to multiply by division (Fig. 32). Such evidence is in itself by no means conclusive, but the case seems to be established decisively in some species by the fact that at certain stages of the life-history true individualized nuclei may be formed by aggregation or growth of the chromidial granules and may later in their turn give off such granules or break down into them (Fig. 343). The conviction has thus become general among protistologists and cytologists that even among the simplest of known organisms the cell always contains nuclear substance ("chromatin" or a related substance), whether in the form of an individualized nucleus or of a scattered nuclear system. Whether the latter can be called a "nucleus" or not is a question of definition. In principle, however, there seems to be no present justification for admitting the existence of "Monera" in Haeckel's sense.[1]

It is therefore highly probable that a chemical and morphological differentiation of the active cell-substance into cytoplasmic and nuclear components is characteristic of all cells *as they now exist* and is necessary to their continued life. This result, primarily based on morphological grounds, is strikingly borne out by physiological experiments on living cells. A fragment of a cell deprived of its nucleus may for a considerable time live and manifest the power of coördinated movement (*e. g.*, in ciliates or rhizopods, p. 657); but it has lost the power of assimilation, growth and repair, and sooner or later dies. The operations of destructive metabolism may continue for a considerable time in the absence of a nucleus; those of constructive metabolism quickly cease with its removal. Strong ground is thus given for the conclusion that the nuclear substance plays some part in the constructive and formative processes of the cell; and this is one of many reasons why the nucleus has come to be widely regarded as a primary factor in growth, development and heredity. There is reason, therefore, to believe that the differentiation of the active cell-substance into cytosome and nucleus is in some manner and degree an expression of

[1] *Cf.* Doflein, '16.

the dual process of metabolism, constructive and destructive, that lies at the basis of all life.

In addition to the nucleus, the cytosome often contains a structure known as the *central apparatus* or *microcentrum* of which the most essential

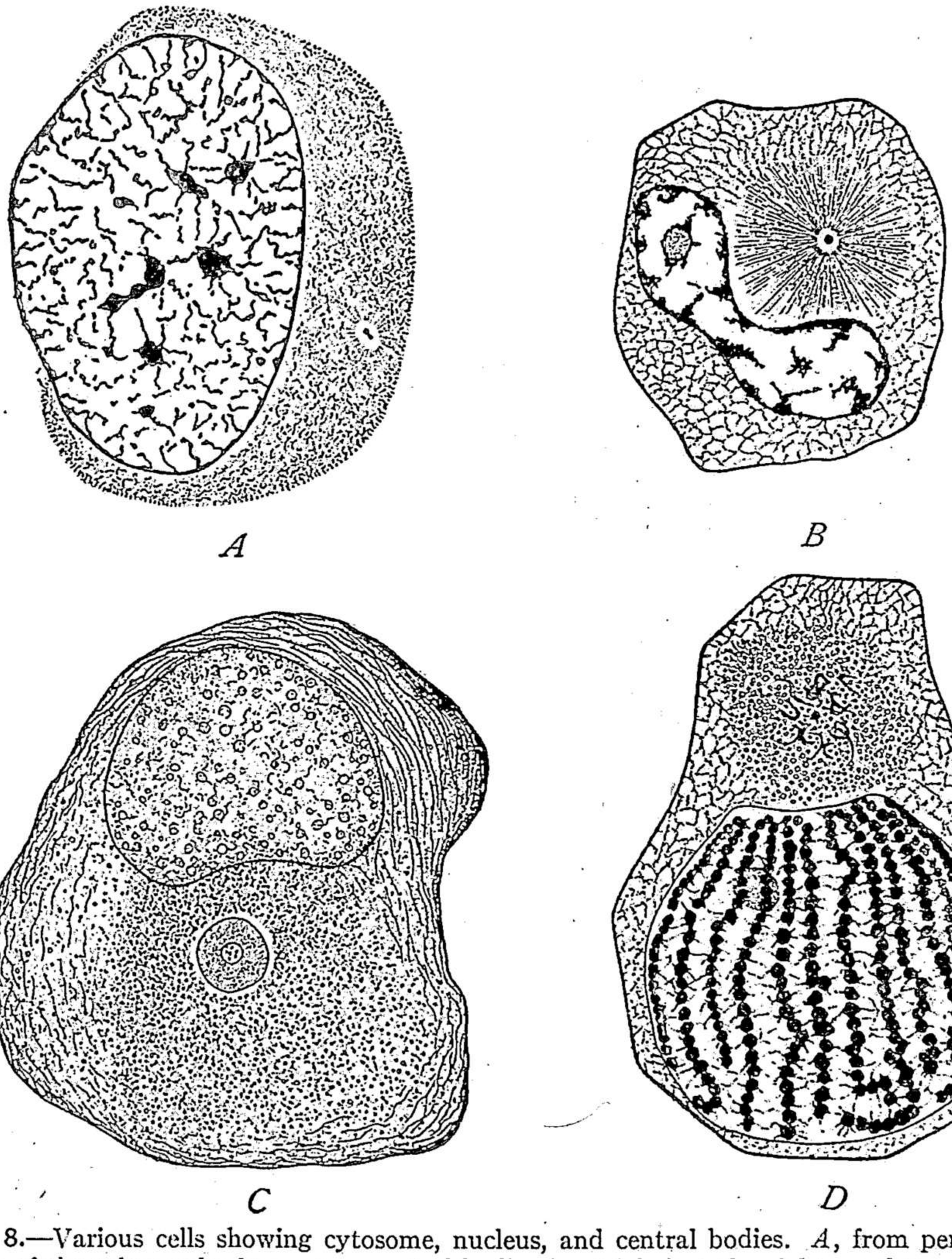
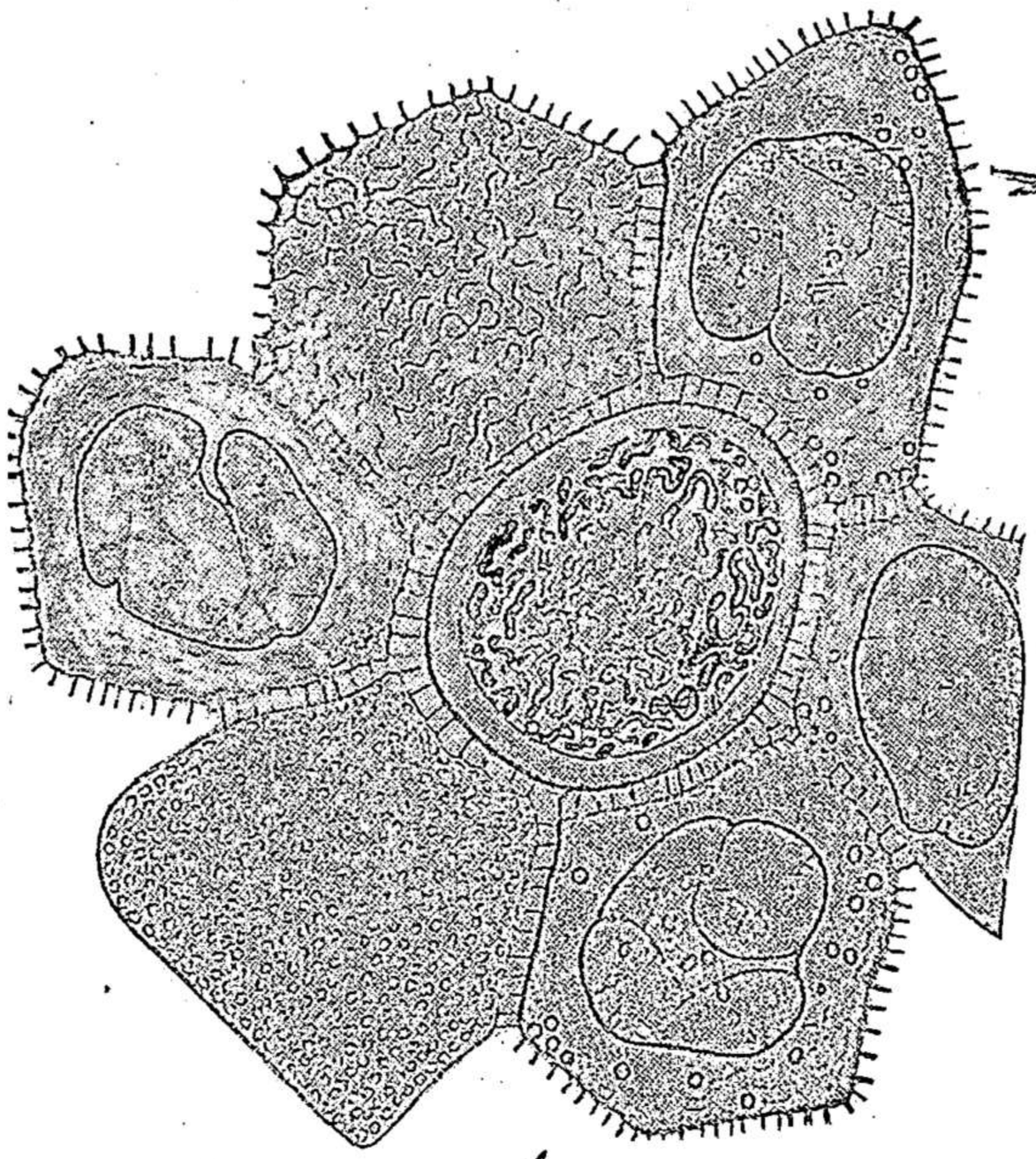


Fig. 8.—Various cells showing cytosome, nucleus, and central bodies. *A*, from peritoneal epithelium of the salamander-larva; two central bodies (centrioles) at the right; nucleus showing net-knots (FLEMMING); *B*, Spermatogonium of frog, aster containing one centriole, nucleus with a single plasmosome (HERMANN); *C*, Spinal ganglion-cell of frog, sphere near the center, containing a single centrosome with several centrioles (LENHOSSÉK); *D*, spermatocyte of *Proteus*, nucleus in the spireme-stage, granular sphere (idiozome) containing a centriole and rod-shaped Golgi-bodies ("pseudo-chromosomes") (HERMANN).

component is the central body (*centrosome, centriole*) about which as a center arise the *asters* that form a conspicuous feature of many forms of mitotic cell-division (p. 144). The central body possesses in many cases the power of growth and division and retains its morphological identity



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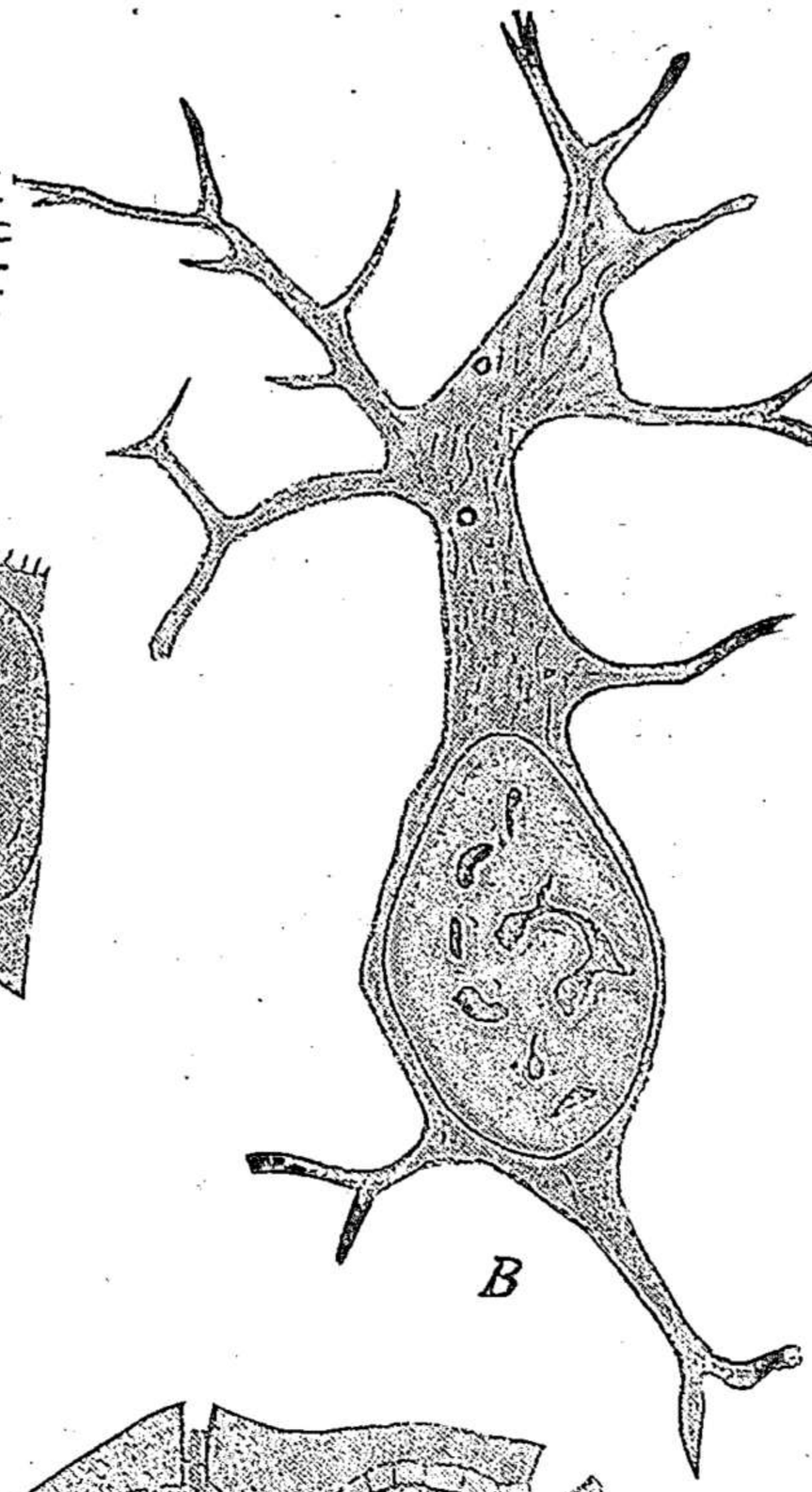

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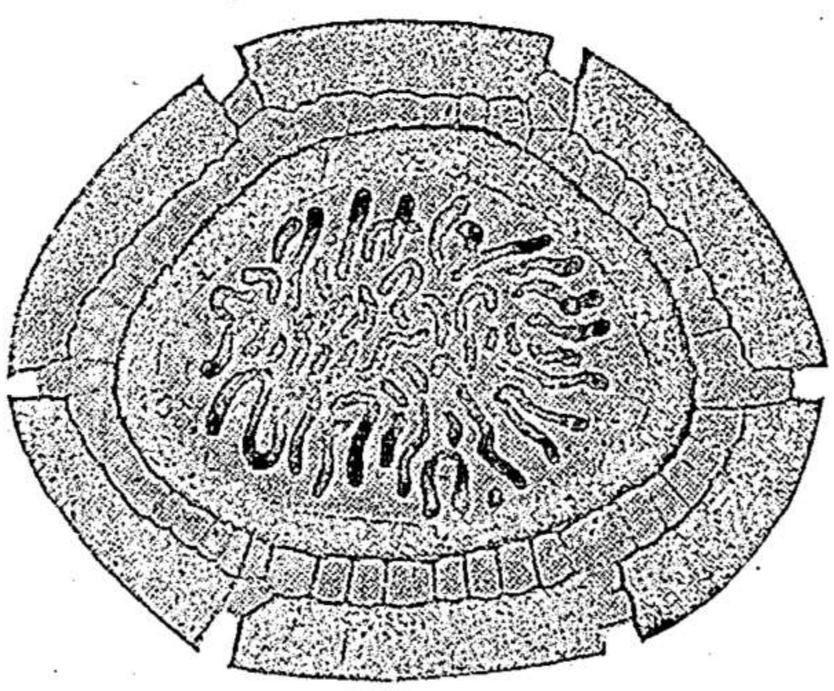

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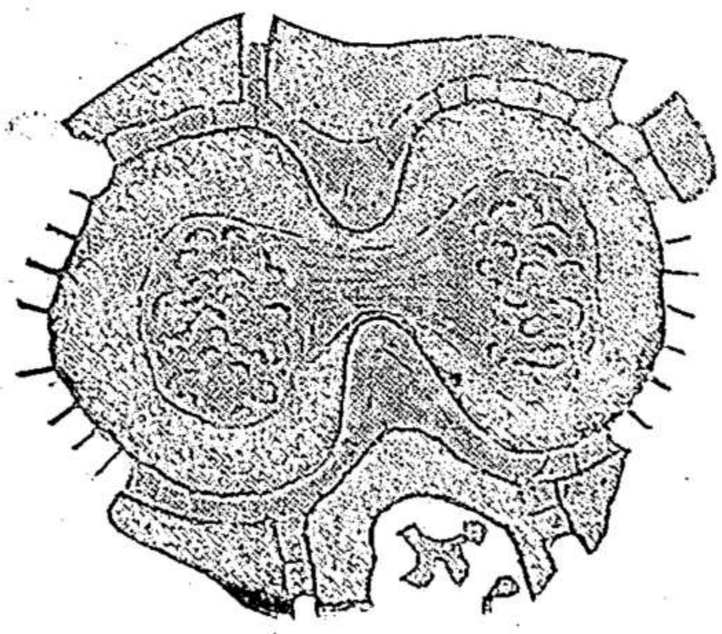

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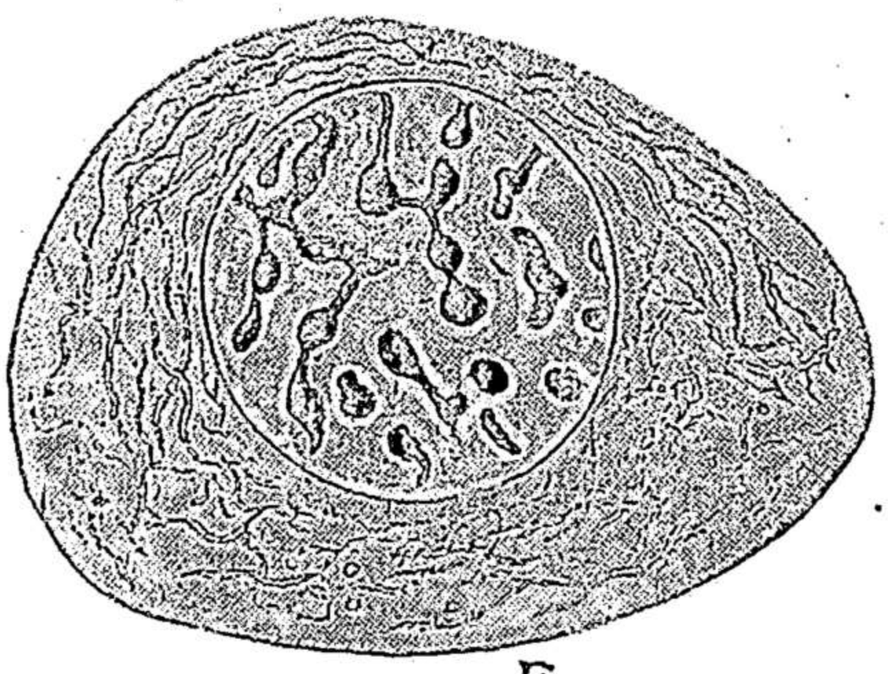

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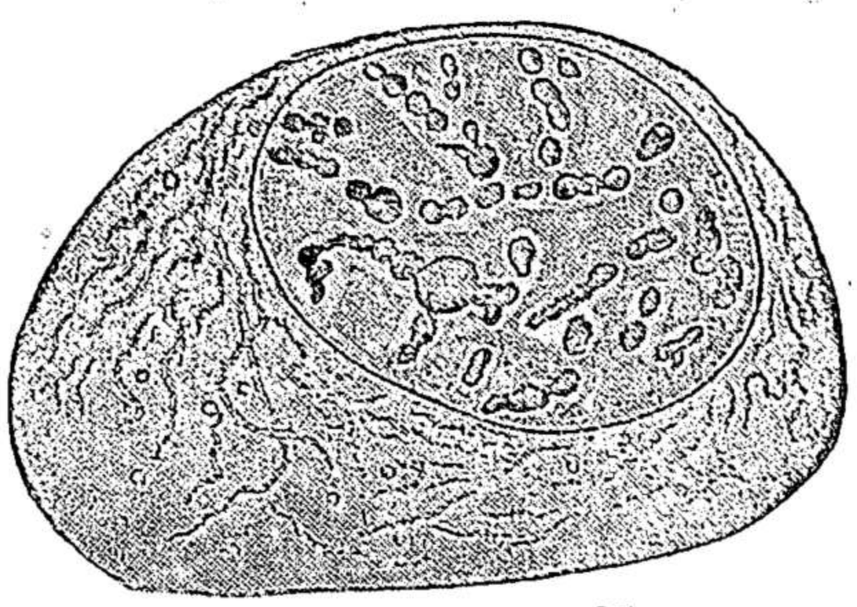

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Fig. 9.—Living cells of salamander-larva (FLEMMING). *A*, group of epidermal cells at different foci; the central cell with nucleus in the spireme-stage; *B*, connective tissue-cell; *C*, epidermal cell in early mitosis (segmented spireme) surrounded by protoplasmic bridges; *D*, dividing cell; *E*, *F*, cartilage-cells with cytoplasmic fibrillæ or chondrioconts.

during the interkinesis or vegetative (non-mitotic) condition of the cell [1] (when it is commonly double). Out of these facts grew the early conclusion of Van Beneden and of Boveri that the central body, like the nucleus, is a permanent and autonomous component of the cell; and Boveri concluded, because of its important rôle in cell-division, that the central body may be regarded as the "dynamic center" of the cell. While there is much to support these conclusions in the case of higher animals they still lack an adequate basis of fact. Though central bodies are present in many lower plants (thallophytes), they seem to be absent in the higher forms; while experimental and cytological evidence has prominently raised the question whether, even in higher animals, they may not under certain conditions be formed *de novo* from the protoplasmic substance (p. 684). The presence of central bodies cannot, therefore, safely be made part of the definition of a cell; and the same is true in respect to various other cell-components, such as the chondriosomes and Golgi-bodies, despite their wide occurrence.

The old definition of Leydig and Schultze, therefore, still holds its own. Since cells are commonly solid bodies nothing could be less appropriate than to call them "cells," and many attempts have been made to find a better name. Beale ('70) long since proposed the word *bioplasm* as a substitute for "protoplasm," at the same time suggesting the appropriate term *bioplast* to designate the living part of the cell (protoplasm and nucleus). This is exactly equivalent to Hanstein's "protoplast" [2] or Sachs's "energid" [3] and seems a better term; but none of these words has thus far become generally current, though Hanstein's term is increasingly used, especially by botanical writers. The word "cell" has indeed become so firmly established, largely because of its convenient brevity, that all efforts to replace it by a better one have failed. Probably, therefore, it must be accepted as part of the established nomenclature of science.

II. THE CYTOSOME AND ITS FORMED COMPONENTS

The cell is a complex living system containing many differentiated structural components which will henceforward be referred to as *formed bodies*. Some of these are found only in the nucleus (nucleoli of various types, etc.), others only in the cytosome (chondriosomes, Golgi-bodies), still others in either nucleus or cytosome (central bodies or division-centers). The cytoplasmic components, vary endlessly in nature, origin and function, and it is difficult to classify them logically. Only those of more general occurrence and significance will here be considered.

[1] Often erroneously spoken of as the "resting cell."
[2] Hanstein, *Das Protoplasma*, 1880.
[3] Sachs, J., *Flora*, 1892.

1. The Central Bodies, Central Apparatus, Microcentrum

The general term *central body* is applied to a structure which forms the focus of the aster or astral system during mitotic cell-division, and hence is often spoken of as the *division-center*. In many cases this body persists during the vegetative or "resting" period of the cell, and is handed on by division to the daughter-cells without loss of its identity; hence the above-mentioned view of Van Beneden and of Boveri that the central body may be a permanent or autonomous cell-organ which always arises from a pre-

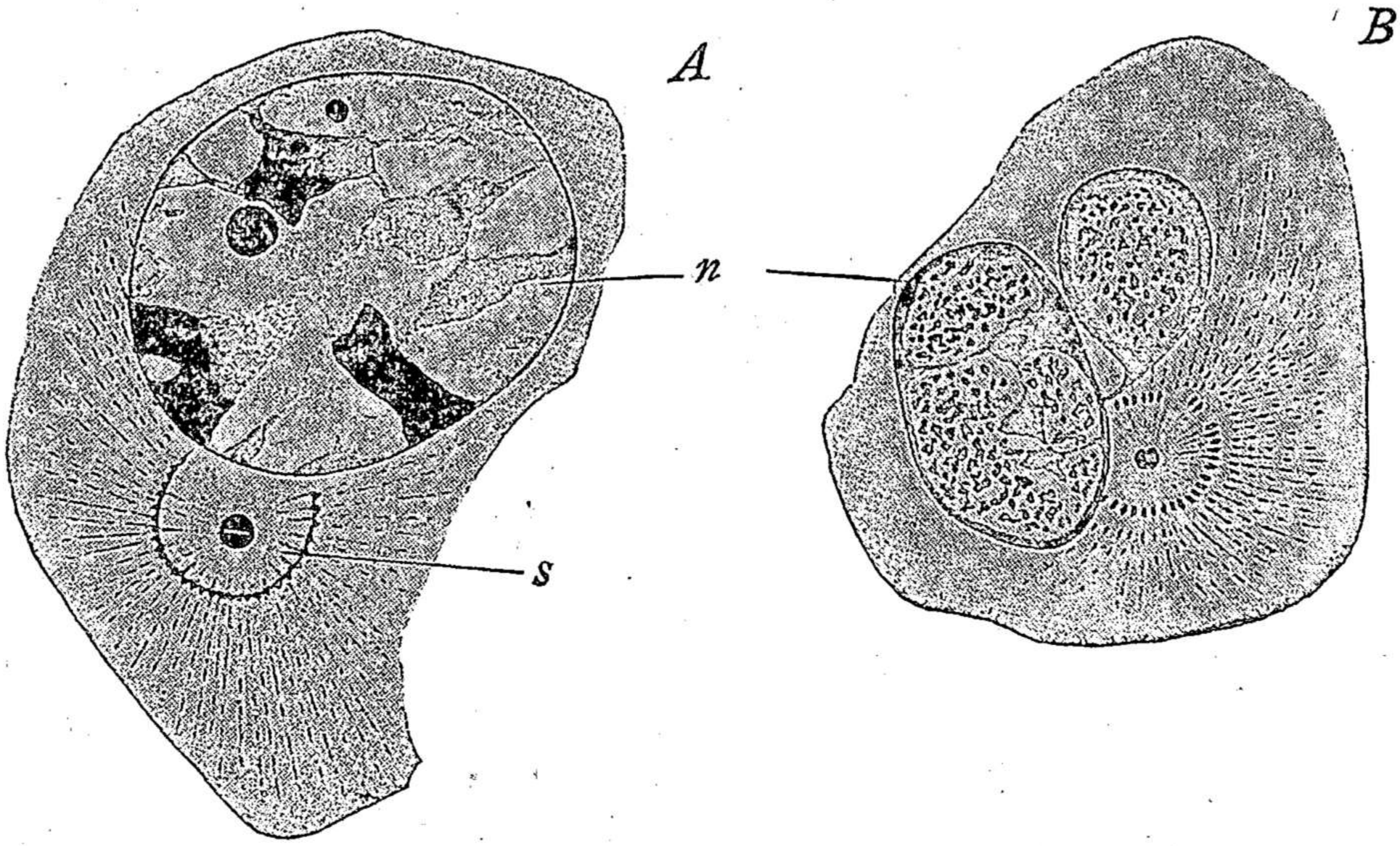

Fig. 10.—Leucocytes of the salamander (HEIDENHAIN). *A*, cell with a single nucleus containing a very coarse network of chromatin and two nucleoli (plasmosomes); *s*, permanent aster, its center occupied by two central bodies surrounded by a microsome-ring; *B*, similar cell, with double nucleus; the smaller dark masses in the latter are oxychromatin-granules (linin), the larger masses are basichromatin.

existing body of the same kind. This conclusion probably went too far; but there is no doubt that the central body often has such an origin.[1]

Van Beneden and Boveri also adopted the hypothesis that the central bodies are of general if not universal occurrence; but this view has not been sustained by later research (p. 150). They are of widespread occurrence in the cells of higher animals and occur in those of many lower plants. On the other hand, with exception of the blepharoplasts (p. 387) they seem to be absent in case of the cells of higher plants (cormophytes) generally. In the resting or vegetative state of the cell the central body is most frequently double, and typically lies in the cytosome; but in some cases it is intranuclear.[2] In the former case the two bodies often lie near the nucleus but may be far removed from it. In epithelial cells generally they commonly

[1] See p. 680.

[2] Exceptionally in Metazoa, commonly in Protista (p. 204).

lie towards the free surface and often very near it, as is typically seen in columnar cells (Fig. 42).

The central bodies and associated structures often form a rather complicated apparatus conveniently designated as the *central apparatus* or *microcentrum*. Its most constant and essential component is the *centriole*, a minute granule or rod, often double, in some cases lying naked in the cytoplasm, more often surrounded by a cytoplasmic investment of various degrees of complexity. In some cases the latter is a rather definite, small rounded spheroid, the *centrosome* (Fig. 8); when larger (Fig. 7) it is often spoken of as the *sphere* (earlier called *attraction-sphere* or *centrosphere*) or in particular cases as the *periplast* or *idiozome*.[1] In practice it is often difficult to distinguish certainly between centriole and centrosome; hence the convenient and non-committal term "central body" which leaves open the question as to its precise homology in any particular case.[2]

The central bodies, in particular the centrioles, are undoubtedly organs of cell-division; but they have a broader significance than this. Even in the vegetative or non-mitotic condition of the cell the central body is sometimes surrounded by radiating fibrillæ to form a more or less definite aster or astral sphere, as is shown conspicuously in leucocytes (Fig. 10) and sometimes on a smaller scale in connective-tissue cells (Fig. 8); sometimes also in the early stages of the animal oöcyte (surrounding the "yolk-nucleus," p. 339), and even in nerve-cells. An interesting example of this is described by Del Rio Hortega ('15) in the cells of Purkinje, where a pair of centrioles, apparently always present, is surrounded by conspicuous, irregularly radiating wavy fibrillæ to form an aster-like body. These cells, so far as known, are not capable of division. The function of the astral formations in these various cases is unknown; but it may possibly be connected with the fact that in the vegetative or non-mitotic phase of the cell the central apparatus often forms a focus about which are aggregated certain of the other formed elements, such as the Golgi-bodies and chondriosomes (p. 329); and in the

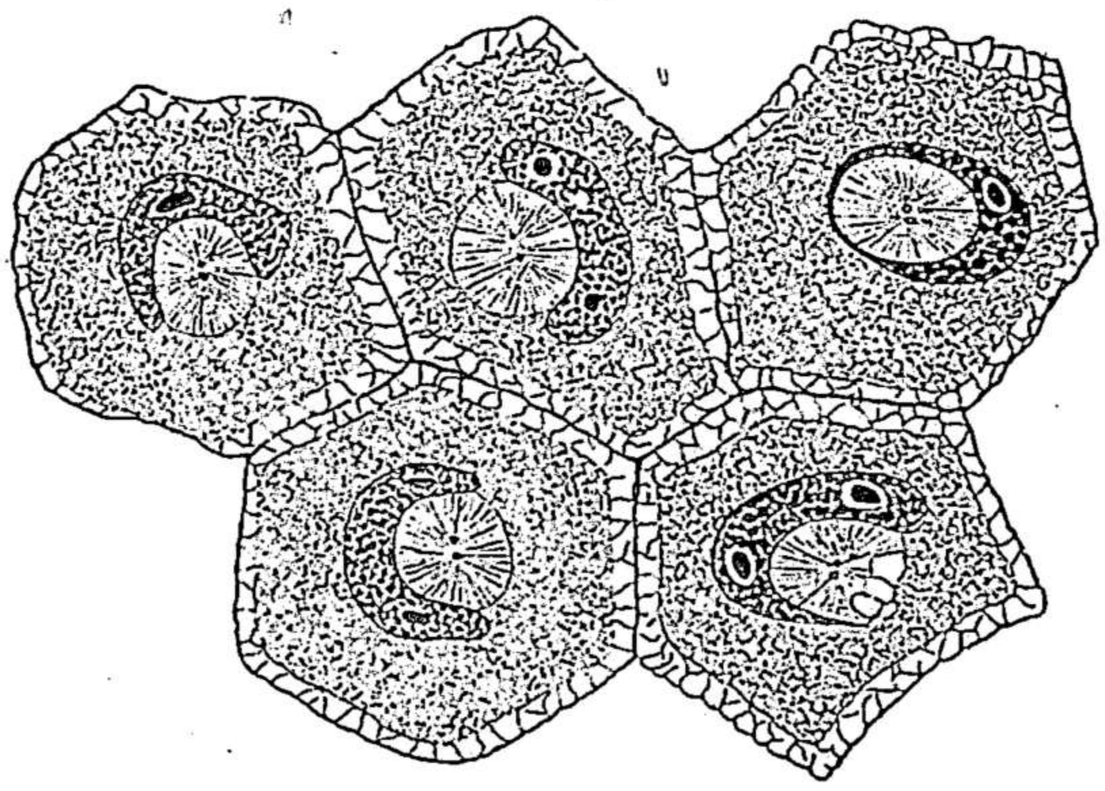

Fig. 11.—Group of cells from the pharyngeal epithelium of the tunicate *Salpa*, showing the radiate sphere and central body lying in a nuclear bay (BALLOWITZ).

[1] For a more critical account see p. 672.

[2] See especially Flemming, '91a, Meves, '02, Boveri, '00, etc. The word "centrosome" has been widely employed especially in the botanical literature as a general term for central body; but this is undesirable. See p. 673.

earlier stages of the animal egg it seems typically to form the original center of the yolk-formation (p. 339). In the early stages of maturation of the germ-cells the central bodies lie at or near that pole of the nucleus towards which the nuclear threads are polarized (Fig. 149), and hence may play a part, if only indirectly, in the conjugation of the spireme-threads during synapsis (p. 550). Again, in the formation of the motile sperms of both plants and animals in many cases the centriole plays the part of a basal body or *blepharoplast*, from which grows forth the axial filament of a flagellum or cilium; [1] and the same seems to be the case also in the flagellated cells of sponges and in many flagellated protista. In some cases, however, the blepharoplasts are quite separate from the centrioles; and it is probable that the centriole may be composed of two closely associated components which may appear as separate bodies. In any case the facts enumerated above show clearly that the central bodies are concerned in many cell-activities that have no immediate connection with cell-division.

2. The Cytoplasmic Granules

Granules are among the most characteristic, widespread and varied of the cytoplasmic formed bodies and have attracted the close attention of cytologists from an early period. They are commonly suspended in a clear, apparently homogeneous and more or less viscid ground-substance or *hyaloplasm*. They vary widely in size, number, staining-reactions, mode of origin and physiological significance, and in many of these respects often show periodic changes correlated with the cyclical activities of the cell, as is typically shown in gland-cells (p. 37). The smallest of the granules graduate down to the limits of true microscopical vision, and the ultra-microscope (p. 33) makes it certain that granules still smaller lie beyond those limits (p. 61). In some cells the cytosome is closely crowded with granules, *e. g.*, the yolk-granules of the animal ovum; in others (as in ectoplasm of many Protozoa), they are so small or few as to be nearly or quite invisible. Such protoplasm is commonly spoken of as "hyaline," because of its glass-like transparency and apparent homogeneity. The larger granules (for instance yolk-granules) vary widely in physical consistency, sometimes being solid or semi-solid bodies, in other cases liquid drops which may readily fuse together when brought into contact.[2] It is probable that similar differences exist among the more minute granules, perhaps among those that are of ultra-microscopical size, and that these too may be either of solid or liquid nature.

[1] In some of these cases the centriole has the form of a rod or V (p. 357).
[2] *Cf.* Wilson, '99.

The classification and terminology of the granules is a difficult matter, involving many disputed questions of fact and of theoretic interpretation. Many of them are relatively passive bodies which belong to the "metaplasmic" or "paraplastic" products of the active protoplasm; examples of these are starch-grains, yolk-granules, and minute drops of fatty or watery liquid. Some kinds of granules, however, such as the various forms of plastids, the centrioles, perhaps also the mitochondria and Golgi-bodies (pp. 45, 48) belong to the more active elements of the protoplasm and in some cases (plastids, centrioles) are self-perpetuating by growth and division.

The structural relation of the granules to other formed elements in the protoplasm (such as fibrillæ, astral rays, alveolar structures, etc.) has given rise to much controversy. When fibrillar formations are present the larger granules and many of the smaller ones are independent of the fibrillæ, *i. e.*, they are *inter-filar* in position. An important group of observers, however (Flemming, Van Beneden, Heidenhain, Altmann, Retzius, and in a measure Benda), have described the protoplasmic fibrillæ as containing small "intra-filar" granules, more or less definitely aligned in linear series so as to give the fibrilla an appearance of segmentation. A similar conclusion, as will later appear (p. 906) is still more strongly suggested in case of the spireme threads that appear in the nucleus of the cell at the time of cell-division (p. 121).

No claim of logical consistency can by made for the following grouping of the granules. It is offered only as a convenient way of defining certain features of the current terminology.[1]

a. Microsomes. This term, at present of only vague and hardly definable meaning, is significant only in the light of its history. By its author (Hanstein, 1880), it was applied to the granules in general, as seen in living protoplasm, in contradistinction to the clear, homogeneous ground-substance or *hyaloplasm* in which they lie, and in this sense it was used by many observers of living protoplasm. In the meantime the word came into general use as applied to the smaller granules seen in fixed (coagulated) and stained preparations, and in particular those supposed to belong to the active protoplasm as distinguished from passive "metaplasmic" storage-granules. More precise subsequent studies showed that these small granules are of various specific types which came to be designated by special names, such as "chromidia," mitochondria," etc. The meaning of the original word "microsome" was thus progressively narrowed until it became a non-committal term, applied to any small granules that could not readily be assigned

[1] A valuable discussion of the granules is given in Heidenhain's great work, *Plasma und Zelle*, ('07, '11), and in Arnold's book on *Plamastrukturen* ('14). See also Schlater ('11), Retzius ('14), Schreiner ('16) and Meves ('18).

to a place in a more specific category. In this vague sense the word is still commonly employed, usually with more or less of a tacit assumption that microsomes form a constant and characteristic component of the active protoplasm. It was apparently with this in mind that some writers proposed to restrict the term to "true" or "intra-filar" microsomes, that form an integral part of the so-called "cytomitome" or cytoplasmic fibrillar system,[1] as was earlier described by Van Beneden, Altmann and many others (Fig. 23). This, however, hardly seems justified by the history of the term, while many observers have also described the "microsomes" that are scattered along the fibrillæ (*e. g.*, astral rays) as adherent to them rather than forming an integral part of their substance. In point of fact, however, the word is commonly employed in a looser and broader sense, as above indicated.

The granules called microsomes are of small size and in many cases graduate down to a minuteness lying at the furthest limits of microscopical vision.[2] They show variable staining-reactions, being in some cases strongly basophilic (p. 87), in other cases oxyphilic, and they appear to be of proteid nature; but their extreme minuteness makes difficult the decision of this question. They have been assumed by some observers to be persistent structural elements, multiplying by fission (*cf.* the "mitochondria"); by others to be derived from the nucleus (*cf.* "chromidia"); by still others to form *de novo* out of the apparently homogeneous hyaloplasm in which they lie, or by the growth of ultra microscopical particles suspended in it. [3] The difficulties here encountered are increased by the fact that minute granules of this type may readily be produced as artificial coagulation-products of an originally homogeneous medium, such as filtered egg-albumin or a solution of albumose. All studies of the granules based on fixed and stained sections must therefore guard against this source of error.

[1] See Heidenhain ('07, p. 476); also Retzius ('14).

[2] Microscopical measurements are usually given in *microns*, or thousandths of a millimeter (1μ = .001 mm.). Dimensions of still smaller order are given in sub-microns or millionths of a millimeter (1 $\mu\mu$ = .001 μ = .000001 mm.). The lower limit of microscopical vision in the ordinary sense of the term (*i. e.*, the limit of resolving power, or capacity to differentiate between two separate objects) was shown by Abbe to be between 200 and 400 $\mu\mu$, a limit fixed by the wave length of light, which in the visible spectrum lies approximately between 450 $\mu\mu$ and 760 $\mu\mu$. Particles lying nearer together than half this distance cannot be distinguished as separate bodies; or, to state the matter differently, particles less than about 200 $\mu\mu$ in diameter cannot be seen as such, since they form no true image, and this may be taken as the practical working limit of the ordinary microscope under the most favorable conditions. (See Barnard, '19.) Particles of these dimensions are said to be ultra-microscopic. Though such particles are invisible as such, their presence may readily be detected by means of the ultra-microscope (*cf.* p. 720) which makes visible the diffraction-images produced by powerful reflected light. By this method it is said that particles as small as 5 $\mu\mu$ in diameter can be distinguished (Hatschek). High powers of the ordinary microscope, as usually employed (*e. g.*, the oil-immersion apochromatic objective of 1.5 mm. with compensation lenses 6–12, Zeiss) give good definition up to 2500–3000 diameters or somewhat higher, but beyond this point, more is lost in definition and illumination than is gained in enlargement.

[3] P. 74. Altmann ('94), Wilson ('99, '23), Heidenhain ('07, '11), etc.

Evidently, the term "microsome," despite its historical priority, has now no definite or generally accepted meaning. It is no more than a convenient synonym for the non-committal phrase "small granule." As such it may often be used with advantage in a purely provisional descriptive sense, provided that it carry no implication as to the specific nature of the bodies thus designated.

b. Mitochondria. Many of the granules now designated by this name were described as "microsomes" by the earlier observers—the term "mitochondria" was indeed first applied by Benda ('98) to granules in the sperm-forming cells that were long ago described as "cytomicrosomes" by La Valette St. George ('86). Since identified as a specific type of granules that occur in nearly all kinds of cells, they have been brought into especial prominence in recent years through the researches of Benda, Meves, Duesberg, Regaud, Guilliermond and others who have ascribed to them an important rôle in histogenesis and heredity. Since they belong to the more general category of the *chondriosomes*, which are considered under another heading (p. 45), they will here be only briefly mentioned. These granules are typically of rather small size, but sometimes very minute, and show the cytological characters (solubilities, staining reactions, etc.) of the chondriosomes generally (p. 45). They are typically separate, being scattered separately through the protoplasm, but according to Benda and a few other observers they may sometimes become aligned in linear series to form fibrillæ known as *chondriomites* (Fig. 12). One of their characteristics appears to be a great plasticity of form; they may elongate to form homogeneous rods or even fibrillæ (*chondrioconts*, p. 46) while the latter may in turn break up into granules, as observed in cultures of living cells *in vitro*. Gradations between the extreme forms are also commonly seen in sections.

In dividing cells the mitochondria are not in or attached to the astral rays but lie between them, and they do not extend into the spindle, though they may closely surround it. They possess remarkable powers of multiplication, and by some observers (Benda, Meves, Duesberg) are believed to be self-perpetuating by division; this conclusion still rests, however, on insufficient evidence. Their possible physiological significance is considered beyond (p. 47).

c. Chromidia or Chromioles. By this term have been designated minute basophilic granules supposed to be derived originally from the nucleus, or (as in various bacteria, rhizopods and flagellates) to form a scattered or distributed nucleus. Much confusion still exists in regard to the relation between them and the mitochondria and other forms of granules. At one time the term "chromidia" was applied in a loose way to many kinds of

basophilic granules in the cytosome with the implication that they are composed of "chromatin" and are presumably of nuclear origin. Later researches proved that in case of the Metazoa many of the granules formerly

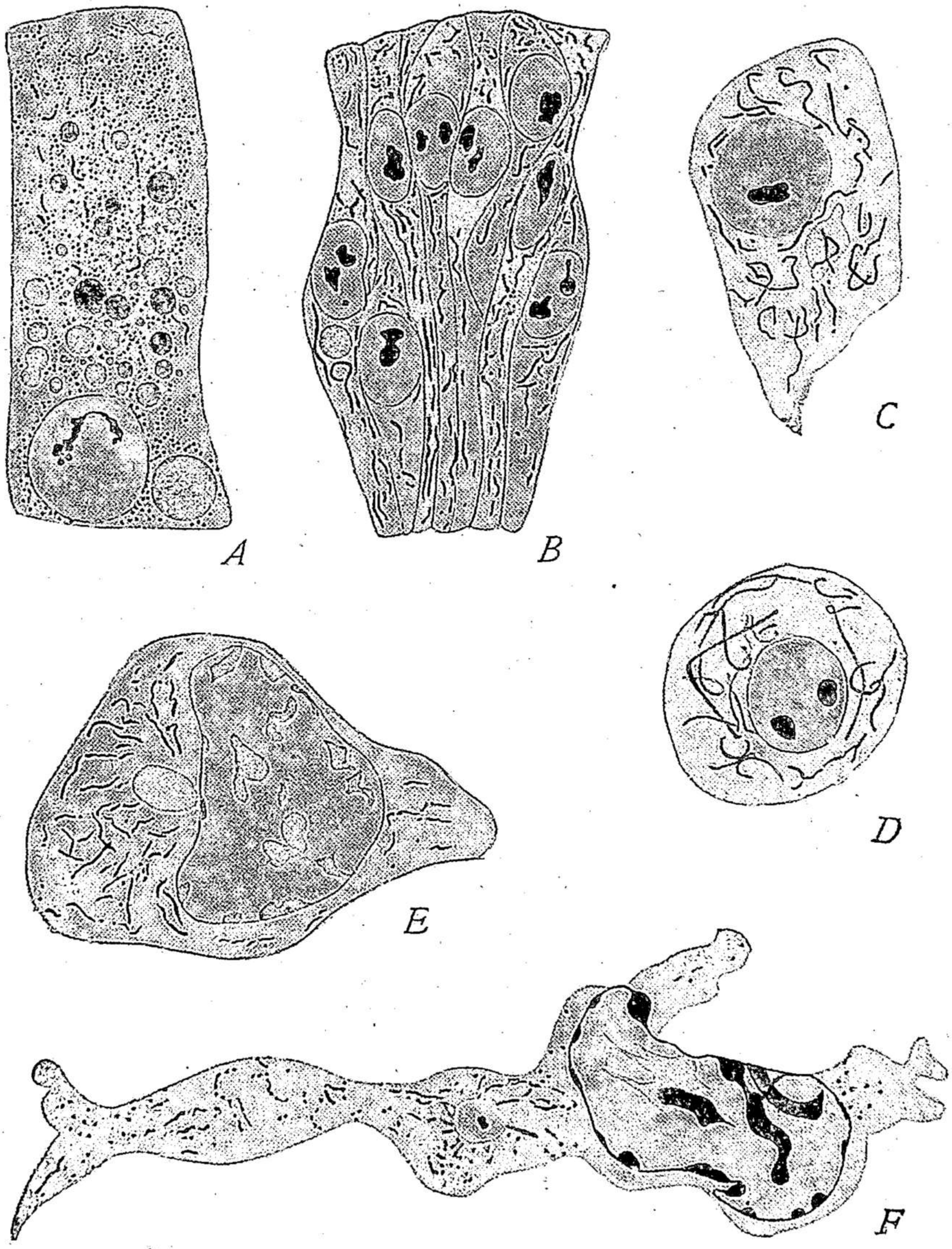

Fig. 12.—Chondriosomes in embryonic cells (MEVES).

A, entoderm-cell, chick of 27 hrs., mitochondria and chondriomites; *B*, group of cells from medullary tube; *C*, cartilage-cell, chick-embryo; *D*, embryonic erythrocyte, chick; *E*, leucocyte, salamander-larva; *F*, wandering cell of same.

described as "chromidia" are mitochondria and have no direct connection with the nucleus, while others are of doubtful origin. It has now become clear that the term chromidia should be strictly reserved for granules known to be of nuclear origin (or to represent a scattered nucleus); and it is at

present an open question whether chromidia as thus defined exist in the cells of higher organisms (p. 700).

The case is different in the Protista, where the researches of R. Hertwig, Schaudinn, Calkins, Dobell, Schaxel, and many others seem to have placed the facts beyond doubt. These granules show the same general reactions as basichromatin (p. 88), staining intensly with basic dyes such as safranin, gentian violet, or hematoxylin, resisting peptic-hydrochloric digestion, and being attacked by nuclease (p. 644). The only certain test of their nature lies, however, in their morphological history, *i. e.*, their derivation from the nucleus or their aggregation to form a nucleus, as is seen in some of the bacteria, rhizopods and flagellates. A further discussion of these granules and their relation to the mitochondria is given in Chapter IX.

d. Metachromatic or Volutin-Granules. The granules thus called are of general interest because of their close general similarity to chromidia, with which they may readily be confused in such groups as the bacteria or blue-green algæ in which the nature of the nucleus has long been in dispute (p. 83). They are of spherical form and variable size, and are found in the protoplasm of many lower organisms (bacteria, spirochætes, cyanophyceæ, various protozoa, fungi and algæ) and probably exist also in higher forms. They are characterized especially by their strong affinity, in fixed material, for various blue or violet basic tar-colors, in particular methylene blue, but also toluidin blue, gentian violet, thionin, etc., in which they commonly stain red or bluish red (hence the term metachromatic). In this respect they are stated to differ from chromidia; [1] nevertheless their basophilic character suggests a chemical relation with chromatin, and they have been regarded by some writers as a stage in its formation. A. Meyer concluded that they consist like basichromatin of nucleic acid combined with an organic base, a view accepted by many later observers. This is supported by the fact that a phosphorus-containing compound is necessary for their development, and Van Herwerden has proved that a nucleic acid compound, readily obtainable from normal yeast, cannot be obtained from volutin-free cultures. [2] On the other hand, it has been shown cytologically by Guilliermond, Dobell and others that the metachromatic granules may coexist in the same cell with a formed nucleus (*e. g.*, in the yeasts), or with chromidial granules. Some writers have therefore concluded that the metachromatic granules are of different nature from chromidia, and presumably represent reserve-material which has no morphological connection with the nucleus. In bacteria they do not take part in the spore-formation (Guilliermond, '08), nor is there other evidence of their morpho-

[1] See especially Meyer ('04, '08), Dobell ('11), Guilliermond ('08, '12), Van Herwerden ('17).
[2] See Reichenow ('16), Van Herwerden ('17).

logical connection with a nucleus. In the blue-green algæ, on the other hand, they enter into the formation of the central body or "karyoplast," and by some recent writers have been regarded as forerunners of the chromioles or chromatin-granules of more highly evolved types of nuclei.[1]

e. Secretory Granules. These granules are of widespread if not universal occurrence in secreting cells whether aggregated to form glands or scattered among other kinds of cells; they are of plastic and transitory nature, sooner or later disintegrating or dissolving to form an important part of the secretion. The constitutent thus produced is often an enzyme, as in glands generally, but may be another substance, such as mucin, or fat. The secretory granules vary widely in size, chemical composition, physical consistency, staining-reactions and internal structure in different kinds of secretory cells, and also during the cycle of activity in the same individual. In their earliest stages these granules are very minute, and have been described by many observers as graduating down to the limits of visibility (E. Müller,

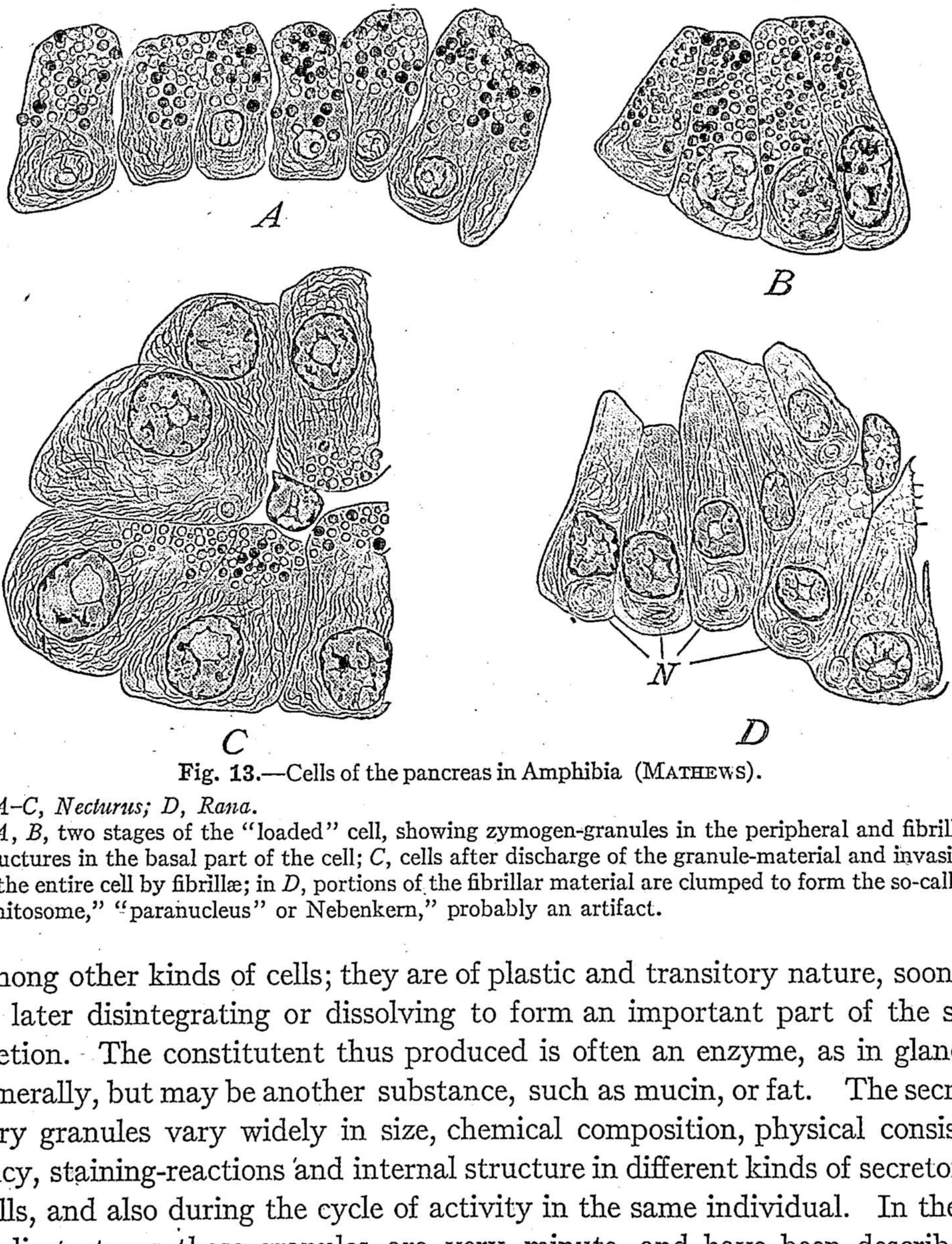

Fig. 13.—Cells of the pancreas in Amphibia (MATHEWS).

A–C, Necturus; D, Rana.

A, B, two stages of the "loaded" cell, showing zymogen-granules in the peripheral and fibrillar structures in the basal part of the cell; *C,* cells after discharge of the granule-material and invasion of the entire cell by fibrillæ; in *D,* portions of the fibrillar material are clumped to form the so-called "mitosome," "paranucleus" or Nebenkern," probably an artifact.

[1] See Acton, '14, Baumgärtel, '20.

Altmann, Heidenhain, etc.). At this time they are hardly to be distinguished from the "microsomes" of undifferentiated protoplasm and show similar staining-reactions. As will later appear, however, many of the more recent observers have concluded that these granules are derived from mitochondria (Altmann, Meves, Regaud, etc.) or from Golgi-bodies (Nassonov, Bowen); still others believe them to arise from extruded fragments of nucleoli (Schreiner in the case of mucous glands). As the primary granules enlarge they often become crowded together so as to produce a honeycomb-like or "pseudalveolar" structure (p. 72) of the protoplasm (Fig. 13). Meanwhile they commonly undergo marked changes of staining-reaction; in the parotid, for example, they stain at first intensely red in acid fuchsin and picric acid, but when fully grown they are yellowish, while the intergranular net is red (Altmann). They may also undergo marked morphological changes, developing a definite structure which differs in different kinds of cells. Ultimately they are converted into the immediate forerunner of the secretory product (zymogen, mucinogen, etc.) and finally break down, or dissolve to form the product itself, thus disappearing as individualized bodies.[1]

f. Storage-Granules and other Forms. Under this heading we may briefly refer to a great variety of granules commonly characterized as "metaplasmic," "paraplasmic," "paraplastic" or "ergastic," since in their fully developed forms they are clearly secondary products of the protoplasmic activity. Examples of these are grains of starch or glycogen, the yolk-granules or deutoplasm-spheres of the animal egg, fat-drops, or the characteristic granules of the leucocytes. They show very wide variations of form, physical consistency, chemical composition, solubility, and staining-reactions. Logically they can hardly be distinguished from the secretory granules; for, like the latter, they are specific protoplasmic products temporarily stored in the cell in the form of discrete bodies destined sooner or later to distintegrate or dissolve, their products often playing a most important part in the life of the organism.

The question of their nature and origin is too large to be taken up *in extenso* at this point. Some of them, such as the starch-grains, are definitely known to be products of plastids (p. 43). Others involve precisely the same problems as those raised by the secretory granules. Fat, for example, is laid down in the protoplasm in the form of small droplets which grow and may coalesce to form larger drops. By the earlier observers the smallest droplets were believed to be laid down by the general protoplasm in the

[1] Heidenhain divides the history of the granule during the secretory cycle into two periods: first a constructive or progressive one during which it grows and assumes its specific character: and second, a degressive or histolytic one in which its substance is transformed into the secretory product and the granule as such finally degenerates ('07, p. 383).

form of vacuoles that might appear at any point. Since the researches of Altmann, however, the opinion has gained ground that fat-synthesis is localized in cytoplasmic corpuscles or granules; but this is not yet decisively demonstrated. In the case of plants these corpuscles have been regarded by some observers as special forms of plastids ("elaioplasts"), analogous to the starch-forming amyloplasts (Wakker, '88); but this too has been disputed. In the case of animals an important group of observers, headed by Arnold ('07, '13, '14, etc.) have followed the lead of Altmann, consider-

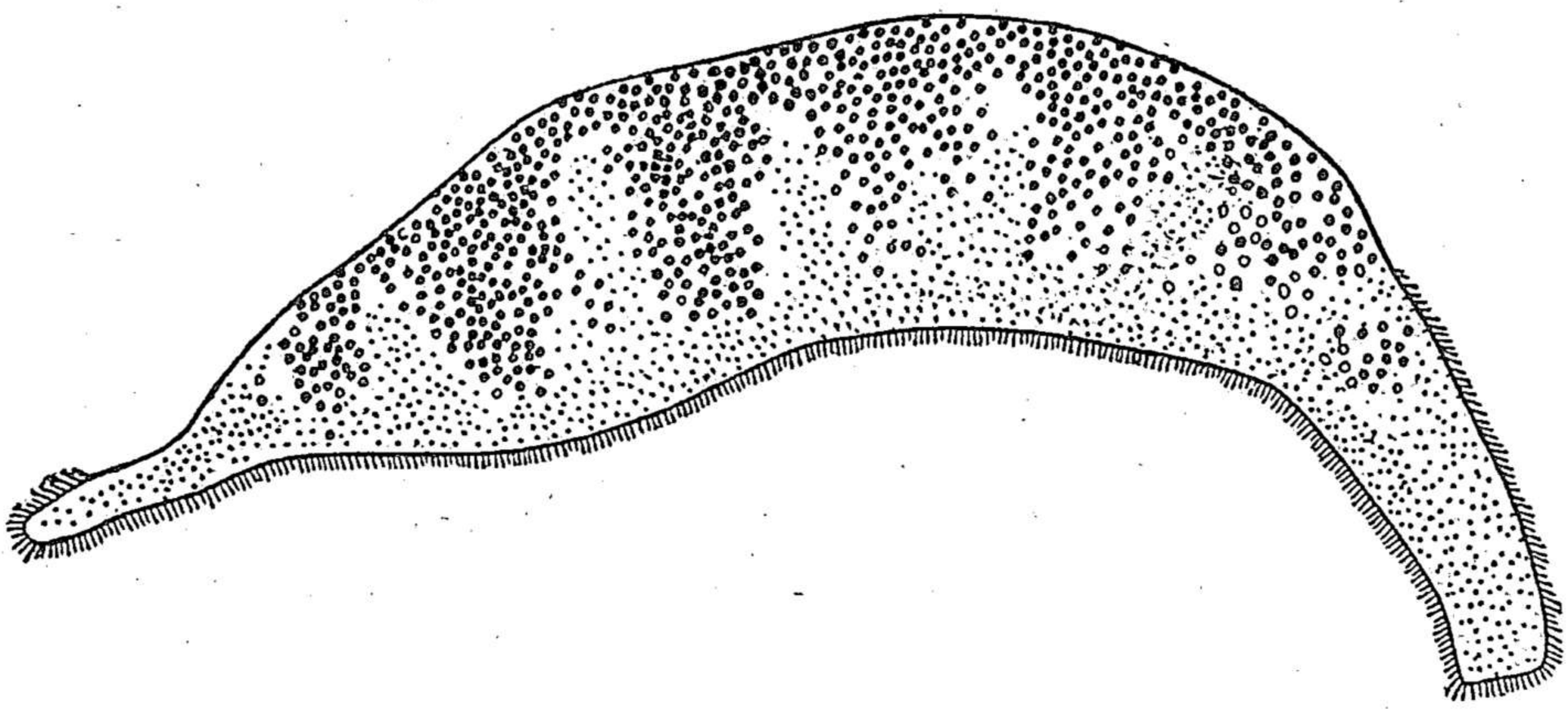

Fig. 14.—A ciliate infusorian, *Trachelocerca*, with chromidial nucleus consisting of scattered chromatin-granules (GRUBER).

ing fat-droplets as products of minute "lipoid granules" which are connected by intermediate conditions with granules which do not show fat-reactions (marked blackening in osmic acid, etc.) and are nearly similar to the protoplasmic "microsomes." These granules have been identified with mitochondria by many modern students of these bodies (Fauré-Fremiet, Dubreuil, etc.) On the other hand, Schreiner, a very competent observer, has recently produced evidence ('15, '16) that the lipoid granules are derived from fragments of *nucleoli* that have been extruded from the nucleus.

Similar uncertainty still hangs over the origin of many other forms of the granules included under this heading, for example, the yolk-spheres or the various forms of granules found in the leucocytes. The former either arise directly from, or are formed in close connection with, a mass of minute granules, at first closely associated with the nucleus and regarded by earlier observers as chromidia extruded from it. It is now generally agreed that these granules are cytoplasmic mitochondria, and strong evidence has been produced that from them arise the yolk-spheres or deutoplasm-granules (Henneguy, Van der Stricht, Loyez, Van Durme, Hirschler, etc.); but here again the evidence is not yet decisive (p. 341).

g. Pigment Granules. Cell-pigments may be diffuse, but most commonly appear in the form of separate and often closely crowded small granules of fairly uniform size. The fact is well-established that some forms of pigment are produced by specific forms of plastids (chlorophyll by the chloroplasts, anthocyanin by the chromoplasts), but it is not yet known whether the

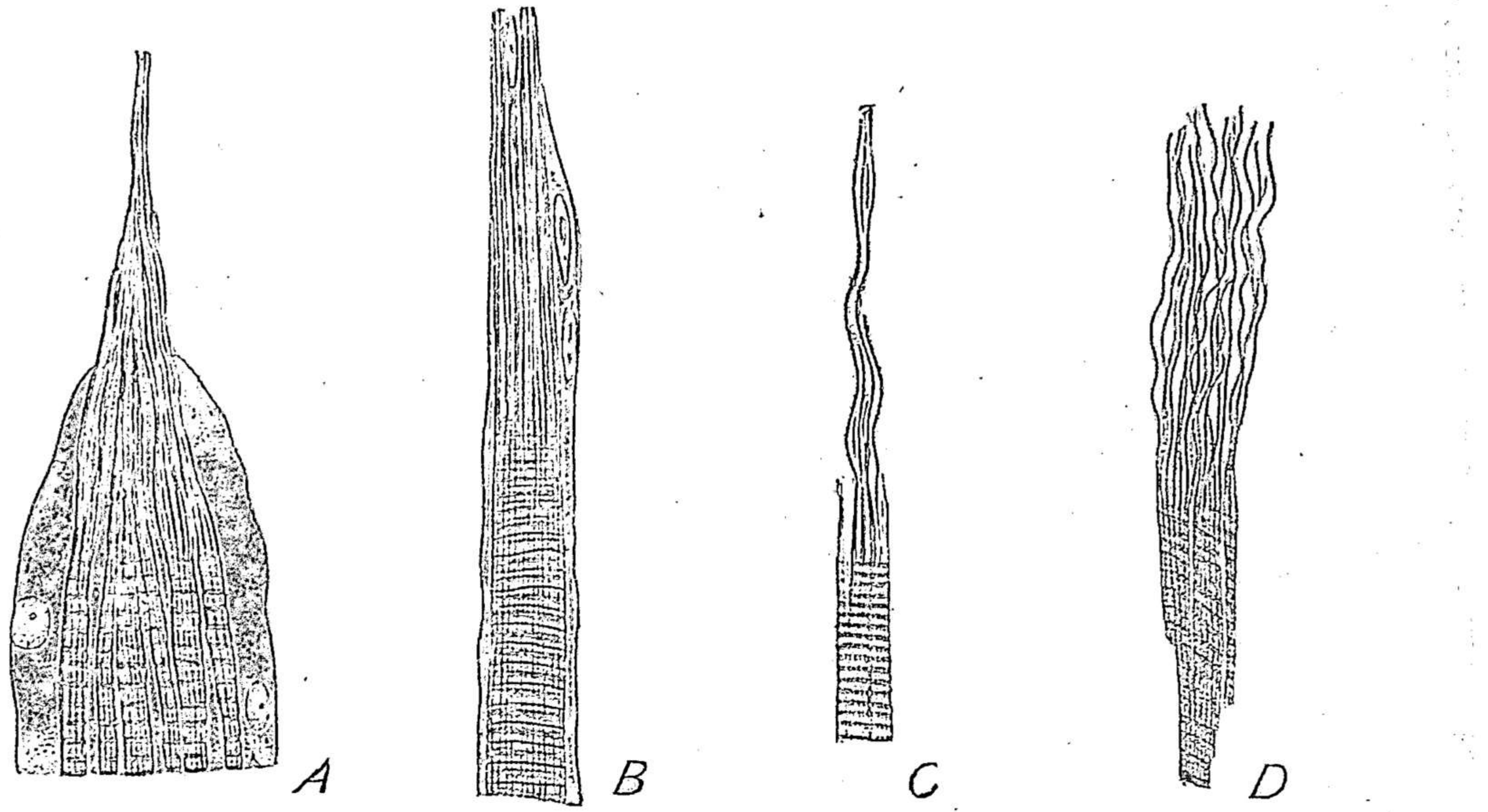

Fig. 15.—Preparations of striated muscle-cells to show longitudinal fibrillæ and their supposed direct connection with those of tendon (O. SCHULTZE).

same is true of pigments generally. A number of modern observers have found[1] that some kinds of pigment-granules arise by the direct transformation of mitochondria or at least arise under their influence. The nature and origin of the pigment-granules thus raises the same general question as that of the secretory or the storage-granules.

3. Fibrillæ

Like the granules, the cytoplasmic fibrillæ are among the most widespread and important components of the cell-substance, and by a prominent school of cytologists, headed by Flemming, have been regarded as an essential feature of the protoplasmic structure (p. 63). They are of many kinds and of varied physiological significance. Among the most familiar forms are the *myofibrils* and *neurofibrils*, characteristic of muscle-cells and nerve-cells respectively. In the striated muscle the myofibrils (Fig. 15) have a complicated structure the precise nature of which has long been a subject of debate.[2] In the nerve-cell they form a more open, net-like structure from which fibrillæ pass out into the axis-cylinder process

[1] See, for example, Meves ('11), Ciaccio ('11), Schridde ('13) on hæmoglobin; also Asvadoura ('13), Prenant ('13) and Duesberg ('15).

[2] For a critical review see Heidenhain's *Plasma und Zelle*, II.

(Fig. 16). Fibrillæ form a widespread and often conspicuous feature of gland-cells (Fig. 13), where they have been assumed by some writers to play an important rôle in secretion (production of the secretory granules) and have been designated as *ergastoplasmic* fibrillæ (Bouin); here as elsewhere, however, their functional significance is still far from clear. Fibrillæ form a conspicuous feature in many forms of epithelia, in particular the columnar epithelia, where the fibrillæ, often closely crowded, commonly run parallel to the long axis of the cell (Figs. 17, 18) in some cases also forming net-like structures.[1] These epitheliofibrillæ have been conjectured to be intra-cellular nerve-endings (neurofibrils), motor elements, analogous to myofibrils, paths of nutritive transportal, etc. The greater number of writers have, however, accepted the conclusion of Nussbaum, Kromayer, Heidenhain ('99) and many others that they are of the nature of supporting or skeletal structures, hence the term *tonofibrillæ* (Heidenhain). This conclusion receives fresh support from the recent work of Del Rio, cited above, which shows that these fibrillæ stain differently from both chondriosomes and neurofibrils and are morphologically distinct from them. In the superficial cells of stratified epithelia the fibrillæ commonly run transversely (parallel to the surface) and it is an interesting fact, observed by a number of earlier histologists and recently confirmed by Del Rio, that many of them traverse the inter-cellular plasma-bridges and thus pass from one cell to another (Fig. 41, *cf.* p. 104).

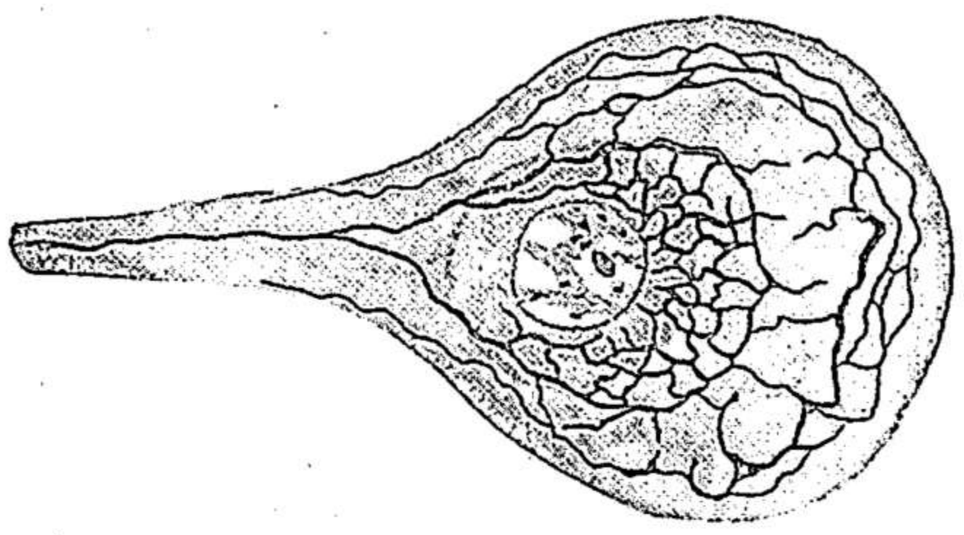

Fig. 16.—Unipolar nerve-cell of earthworm stained with gold chloride, showing neurofibrils and axis-cylinder (SZÜTS).

Other forms of fibrillæ are exemplified by the basal filaments, rhizoplasts, or ciliary roots of flagellated or ciliated cells; perhaps by the so-called spindle-fibers and astral rays in the mitotic figure; and those forms of chondriosomes known as chondrioconts. An especial interest attaches to the latter, which are found in nearly all kinds of cells, because of the conclusion of Benda, Meves and others that they represent the most primitive type of fibrillæ from which in the course of histogenesis arise directly many if not all of the more differentiated forms, such as myofibrils, neurofibrils or glandular fibrils. This view, as will be later shown, is still insufficiently based and must await the test of further inquiry.

[1] The epitheliofibrillæ have been studied by many observers, especially by M. Heidenhain ('99) and more recently by Del Rio-Hortega ('17), who has obtained very striking pictures by the use of Achúcarro's method (tannin-silver impregnation) and offers a valuable discussion of the subject. Preparations made in my laboratory by J. Nonidez show that Del Rio's remarkable figures do not exaggerate the clearness and brilliancy of the preparations.

Many of the forms of fibrillæ mentioned above have been regarded as artifacts or in some cases as optical illusions (Bütschli); and the question here raised remains to a considerable extent unsettled. For example, it is still by no means certain that the archiplasmic fibrillæ (spindle-fibers and

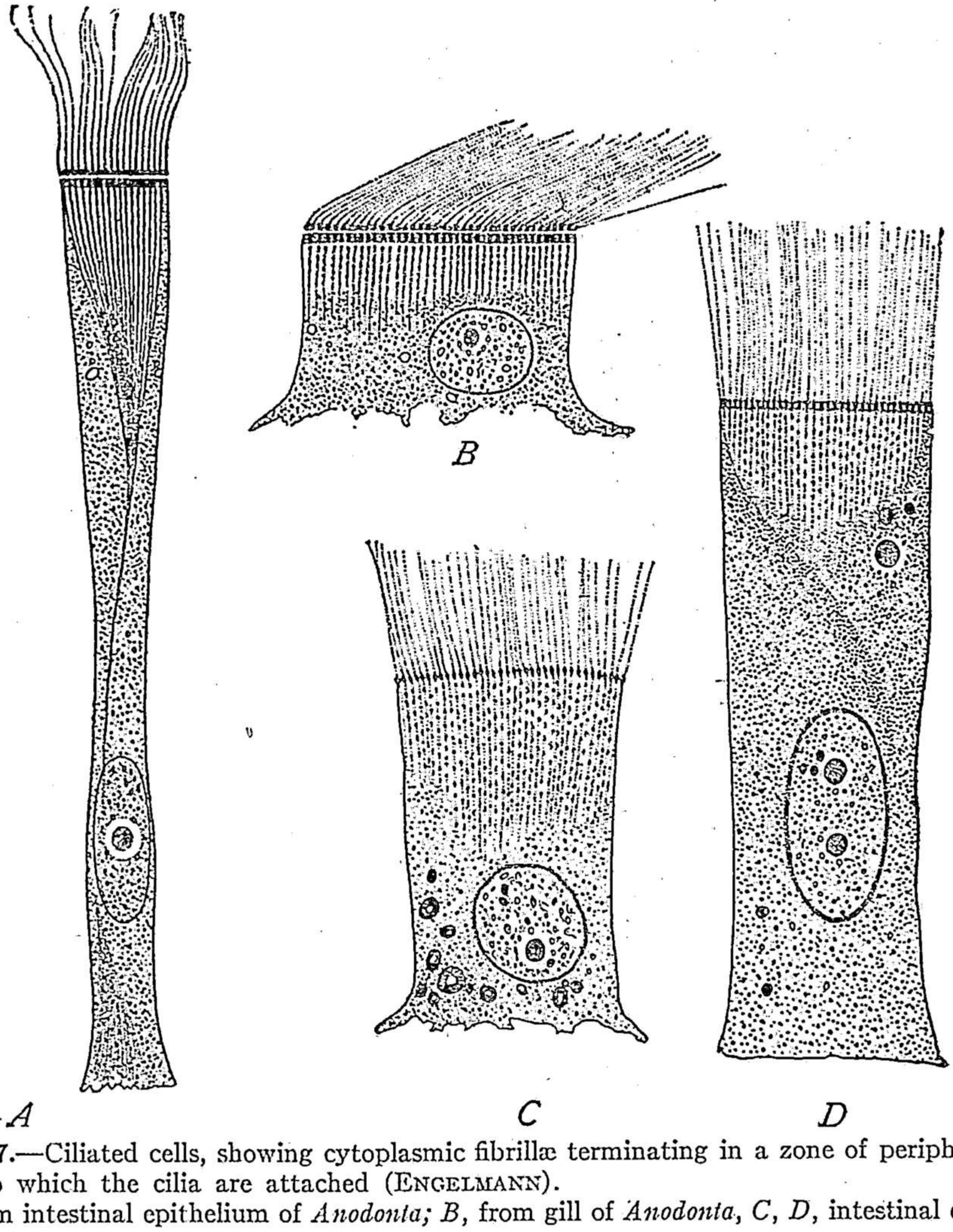

Fig. 17.—Ciliated cells, showing cytoplasmic fibrillæ terminating in a zone of peripheral basal bodies to which the cilia are attached (ENGELMANN).

A, from intestinal epithelium of *Anodonta; B*, from gill of *Anodonta*, *C*, *D*, intestinal epithelium of *Cyclas*.

astral rays) actually preëxist in the living protoplasm; and it has been shown that these and certain other forms of fibrillæ may be closely simulated by the coagulation of homogeneous colloidal solutions, such as filtered egg-albumin or solutions of albumose (p. 65). On the other hand, some kinds of fibrillæ, such as the chondrioconts may clearly be seen in living protoplasm, as was long since shown by Flemming ('82) and confirmed by many more recent observers. The actual existence of fibrillæ as preformed

structural components of the protoplasmic substance can therefore not be doubted. [1]

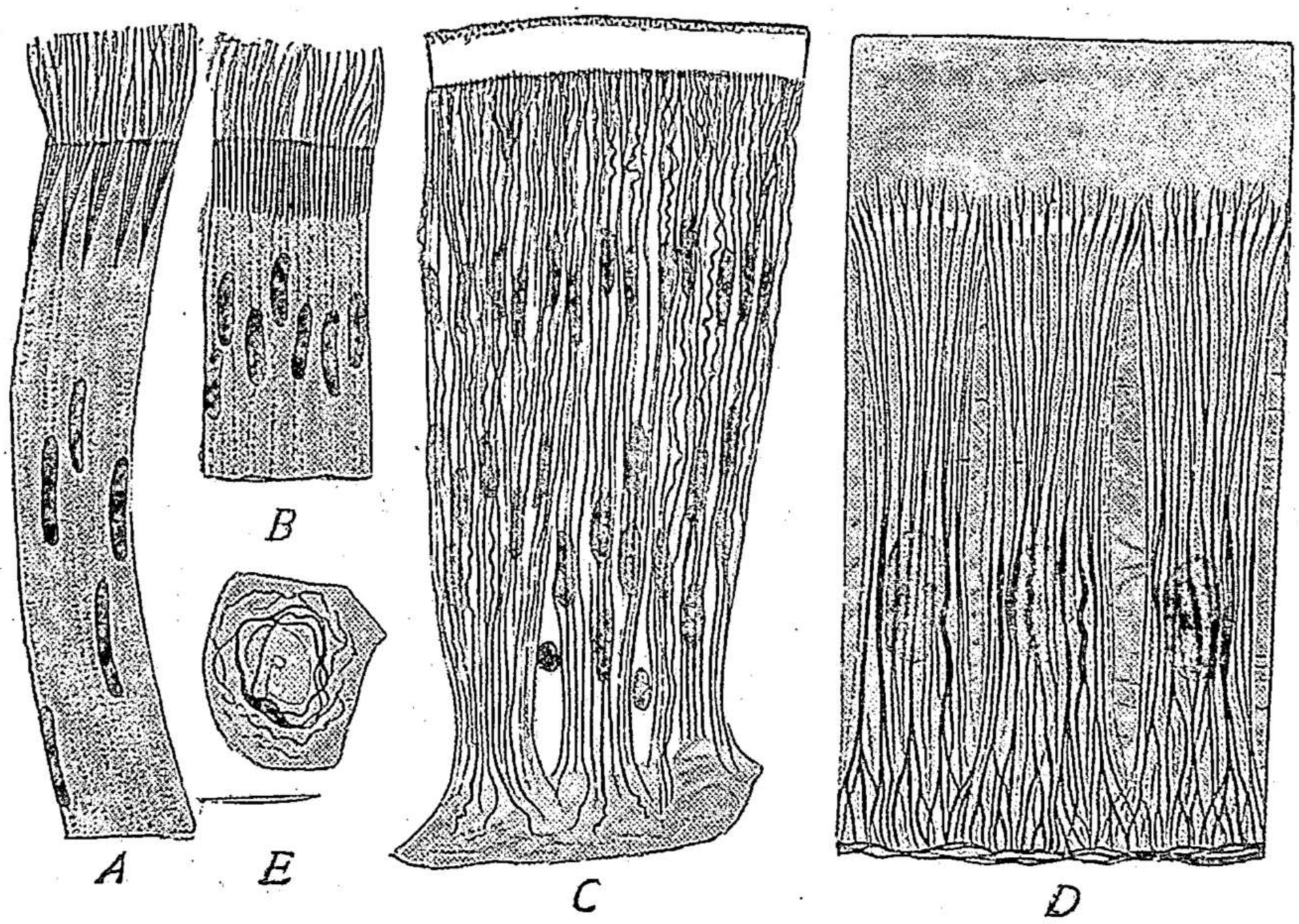

Fig. 18.—Protoplasmic fibrillæ of epithelial cells as demonstrated by the Achúcarro-Del-Rio method of gold-silver impregnation (DEL RIO).

A, *B*, columnar cells, intestinal epithelium of the mollusk *Tapes*, showing basal granules (blepharoplasts, trichoplasts) and basal rods (rhizoplasts); *C*, œsophageal epithelium of *Lumbricus*, with longitudinal fibrillæ; *D*, bucco-pharyngeal epithelium of the snail *Aplysia*; *E*, cell from the deeper epidermis of the toad *Pelobates*. [2]

4. Plastids

These bodies, especially characteristic of the cells of plants, are of general interest because they possess in many cases the power of independent growth and division, and many competent observers have accepted the probability that they arise in no other way. They are usually bodies of definite form, exclusively cytoplasmic, and vary widely in number, form and size; in some cases they are single or few in number (many algæ), in others very numerous (chloroplasts of higher plants generally); they are commonly rounded in form, but may be band-shaped, lobed or irregular. Physiologically they are localized areas of specific chemical transformation, producing characteristic products, such as starch, pigment of various kinds and perhaps fat, and are classified accordingly.

In their least differentiated condition they are small, colorless bodies, known as (1) *leucoplasts*, especially abundant in embryonic tissues but also found in differentiated cells. From the embryonic leucoplasts (themselves possibly derived from chondriosomes),[3] arise various other forms of

1 *Cf.* p. 64.
2 These figures do not exaggerate the clearness with which the fibrillæ appear.
3 See p. 709.

plastids, as differentiation proceeds. In some cases they remain colorless, but enlarge to form (2) *amyloplasts*, which act as centers for the formation of reserve starch in the storage-tissues by the transformation of dissolved carbohydrates (glucose) into solid starch-grains. In other cases the plastid

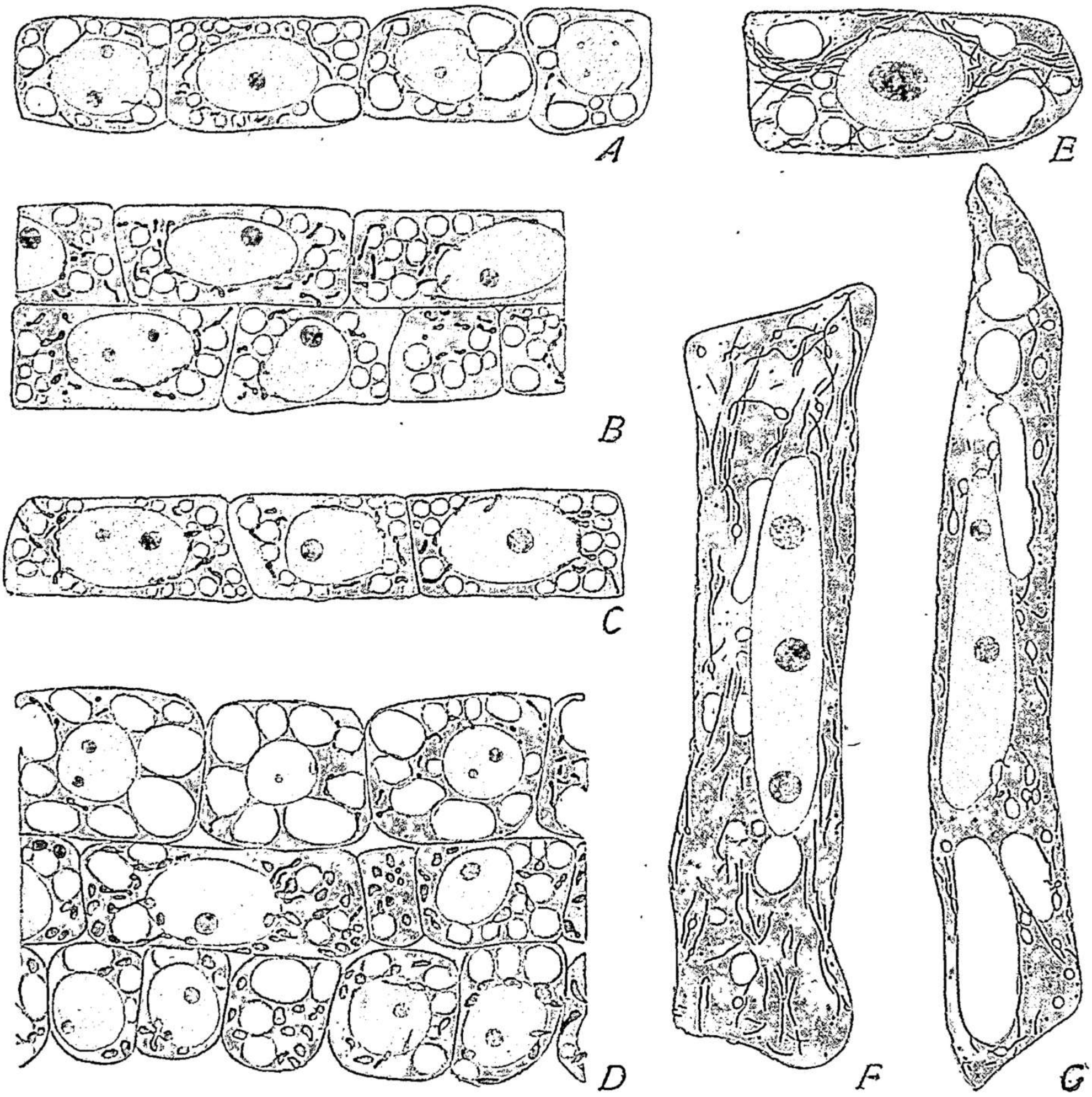

Fig. 19.—Plastids and chondriosomes in seed-plants (MEVES).

A, embryonic cells from young leaf-bud of *Tradescantia*, showing chondriosomes (in black); *B*, cells from meristem at base of older leaf; *C*, *D*, from same leaf, nearer the tip, showing supposed stages of division of the chondriosomes and their transformation into chloroplasts; *E*, embryonic cell from aerial roots of *Chlorophytum*, showing chondrioconts; *F*, *G*, older cells of same, showing formation of starch-grains in the chondrioconts.

develops pigment and becomes a *chromoplast* or *chromatophore*. The most important of these physiologically are (3) the *chloroplasts* or chlorophyll-bodies which are centers for the new formation of starch by photosynthesis (Fig. 19). The so-called stigma or "eye-spot" of various flagellates, zoöspores and plant gametes, a light-sensitive organella, has been regarded by some authors as a special type of chromoplast; and some authors have also regarded as plastids the *pyrenoids*, localized bodies imbedded in the

chromoplasts of green algæ, and serving as localized centers of starch-formation.

Besides the undoubted forms of plastids at least two other types of plastid-like bodies have been recognized by some observers. These are: (4) the *tonoplasts* which, according to DeVries, give rise to the vacuoles, the walls of which they form, and (5) the *elaioplasts*, regarded by Wakker (1888) and his followers as plastids which act as centers of fat-formation. The plastid nature of these two types is, however, somewhat uncertain.

The classical work of Schimper (1881–85) and of A. Meyer (1883) led them to the conclusion that plastids are never formed *de novo* but always by the growth and division of preëxisting plastids and ultimately from the minute undifferentiated leucoplasts of the germ-cells. They were thus conceived as having a persistent individuality and conforming to the general law of genetic continuity, like cells, nuclei or chromosomes (p. 828). The fact is now generally admitted that differentiated forms of plastids, in particular the chloroplasts, multiply in this manner; and in some lower plants the plastids are known to divide regularly at each cell-division (*e. g.*, in *Zygnema*, or *Anthoceros*).[1] No general agreement has, however, yet been reached as to whether plastids may not also arise *de novo* in the cytoplasm.[2] In this respect they are in the same case as the chondriosomes and the Golgi-bodies described below. In recent years strong evidence has been brought forward to show that plastids are of the same nature as chondriosomes (Levitsky, Guilliermond, Meves, etc.) and are actually derived from them in the course of early development (p. 709).

5. Chondriosomes [3]

These bodies, or their products, are among the most characteristic of the formed components of the cytosome and are known to occur in nearly all kinds of cells, among both plants and animals, and everywhere showing the same general characters. They have attracted much attention in recent

[1] Davis (99), 'Kursanow ('11), Scherrer ('14).

[2] *Cf.* Harper ('19).

[3] The term "chondriosome" was suggested by Benda ('04) and brought into more general use by Meves ('08). Meves also suggested the word *chondrioma* to designate the entire chondriosome-content of the cell; but this term, though sometimes convenient, has not been widely employed. Cowdry ('19, '16, and earlier) has urged the desirability of replacing the term "chondriosomes" by the earlier one "mitochondria" (Benda), employed in a more general sense, so as to apply to all the forms later called" chondriosomes." The word mitochondria (thread-granules) seems, however, both etymologically and historically to be most appropriately applied to the granules as such, rather than to other forms which they may assume. Meves ('10) proposed to replace these various terms by new ones containing the component "plasto" (*plastos*, form) because of the important part supposed to be played by the chondriosomes in histogenesis; hence, *plastosomes*, *plastochondria*, *plastoconts*. Many more special terms, such as *chondrioplasts*, *chromochondria*, *myochondria*, etc., are found in the literature. See Glossary; consult also the useful table of terms given by Cowdry ('18).

years because of the questions raised by Altmann, Benda, Meves and their followers concerning their possible significance in histogenesis and heredity; but opinion concerning them is still in a very unsettled state. Morphologically they appear in the form of small granules (*mitochondria*), rods or filaments (*chondrioconts*) and other bodies, many of which were observed by the earlier observers of protoplasm and described under the name of "granules," "microsomes," protoplasmic fibrillæ or "fila," "nebenkerns," etc. In a sense, therefore, we are here dealing with new names for old things.[1] More recent studies have shown that they consist of a specific material, showing definite cytological and michrochemical characters but morphologically highly plastic, so that it may appear under many forms, which are probably to be regarded as only different phases of the same material. The most common of these are separate mitochondria and chondrioconts, both of which may often be observed in the same cell (Fig. 12); and all gradations between them may be observed in sections. Less frequently the mitochondria are aligned in linear series to form *chondriomites;* while in special cases the chondriosomes may enlarge or aggregate to form more massive bodies, spheroidal *chondriospheres* (Fig. 168), or even may give rise to a single body, such as the "nebenkern" of the sperm-forming cells (Figs. 164, 174) or the ring-shaped chrondriosome-body of *Centrurus* (Fig. 169). These bodies often show a differentiation into a more deeply staining cortical and a lightly staining central or medullary substance, which in the sperm-forming cells may give rise to complicated structural patterns (p. 372). All such more complicated forms seem, however, to be secondary and specialized formations which arise primarily from minute scattered granules, rods or threads.

According to M. R. and W. H. Lewis ('14, '15), the chondriosomes as seen in cultures of living cells *in vitro* are almost never at rest, often changing their shape from moment to moment, and also undergoing rapid changes of position. The various morphological forms are readily transformed one into another. In the living cell "granules may be seen to fuse into rows or chains, and these to elongate into threads"; and these are said in turn to anastomose with each other and may give rise to a complicated network which in turn may again break down into threads, rods, loops, and rings.[2] The threads or chondrioconts are, however, stated to arise more commonly by the stretching out of single granules; and it should be added that few other observers have found the threads anastomosing to form networks. These observations, together with the evidence offered by fixed preparations, leave no doubt of the plasticity and polymorphic character of the chondriosomes; though the possibility should be kept in mind that some of the

[1] See Retzius, '14.

[2] '14, p. 331.

changes seen in living cultures may be due to slightly abnormal conditions under which the cells are placed.

The physico-chemical nature of chondriosomes has been the object of numerous researches [1] which indicate that their principal chemical components are phospholipoid and albuminous substances, thus resembling chemically the phosphatids, of which lecithin is an example; and we here find some indication of their reactions to fixing and staining agents. They are soluble in various degrees in dilute acetic acid, ether, acetone, alcohol and other fat-solvents; hence the fact that they are often imperfectly fixed or even destroyed by many of the ordinary fixing agents containing acetic acid, and were often overlooked until a more appropriate technique had been devised.[2] They often darken more or less in osmic acid, though less so than the Golgi-elements, to which they appear to be somewhat related chemically (p. 48). In sections they are stained by various dyes, of which those most frequently employed are iron hæmatoxylin, crystal violet (Benda's alizarin-crystal-violet method) and acid-fuchsin (Altmann's acid-fuchsin picric acid or Bensley's acid-fuchsin methyl-green). In the living state they are stained characteristically by weak solutions of Janus green B. As in the case of so many other cell-components, however, their identification rests less upon their microchemical reactions than on their morphological history.

The broader theoretical interest of the chondriosomes as possibly persistent and autonomous cell-components, which has been urged by Altmann, Benda, Meves, Duesberg, Guilliermond and many others,[3] will be more fully considered later. They play an important part in the formation of the germ-cells (p. 369); during cell-division they are distributed with approximate equality to the daughter-cells (*chondriokinesis*, p. 163). An important group of observers have ascribed to them the powers of independent growth and division, and consider them as of fundamental importance for the process of histogenesis (p. 706), forming the source from which arise many of the more specific cell-components, including the plastids, various forms of fibrillæ, such as the neurofibrils and myofibrils, and a great variety of granules, such as secretory and storage-granules, yolk, fat, pigment, etc. In this direction the chondriosòme-theory comes into close relation with the granule-theory of protoplasm as developed by Altmann and his followers (p. 74).

[1] See especially Regaud ('08), Fauré-Fremiet ('10), Löwschin ('13). Also the general reviews of Kingsbury ('12), Duesberg ('12), E. V. Cowdry ('16, '18), N. H. Cowdry ('17), Guilliermond ('14, etc.).

[2] *E. g.*, Benda's fluid (Flemming's with very little acetic or none, or those of Altmann, Bensley, Regaud and Champy. See Cowdry, *op. cit.* Also Gatenby in Lee ('21).

[3] See Benda ('03), Meves ('07, '08, '18, etc.), Duesberg ('07, '19, etc.), Guilliermond ('14, etc.).

Little is certainly known as yet concerning their specific physiological meaning. Because of their important rôle in the formation of the sperm-tail Benda conjectured that they may be contractile and perform motor functions; but this has found little or no support, nor has the later suggestions of Koltzoff ('06, '09) that they are in the nature of skeletal or supporting structures. Kingsbury ('12) and Mayer, Rathery and Schaffer ('14) seek to connect them with the respiratory functions; but this view also lacks definite support, though it has been favorably regarded by some authors. More promising is the view of Regaud ('09a, '10), who considers the chondriosomes as centers of specific chemical action, like the plastids, which serve to extract, elaborate and fix definite chemical constituents of the protoplasm, hence the term *electosomes*. This view, a modification of that of Benda and Meves, is based on observations which have seemed to show, as above stated, that the mitochondria may actually give rise to other intracellular structures of specific type. To this subject we shall later return in a more general discussion of the theoretical significance of the chondriosomes.[1]

6. Golgi-apparatus, Golgi-bodies, Dictyosomes. ("Internal Reticular Apparatus" of Golgi. "Canalicular System" or "Trophospongium" of Holmgren)

By these various names are designated a group of cell-components, as yet imperfectly known, which show some points of resemblance to the chondriosomes though morphologically quite distinct from them. Like the chondriosomes the Golgi-elements are in considerable degree polymorphic, though always consisting, apparently, of the same specific material. Like the chondriosomes they blacken with osmic acid, but much more intensely; and they show somewhat similar solubilities, being readily attacked by dilute acetic acid and other lipoid-solvents, so that they are often destroyed or imperfectly fixed by reagents containing these ingredients. In these respects they, like the chondriosomes, behave somewhat like lecithin-compounds and may possibly be composed of lecithalbumin (Weigl, '12). The best methods for their demonstration consist in fixation by reagents containing little or no acetic acid and impregnation by metallic silver or osmium,[2] by which they are usually well differentiated from the chondriosomes. Their identification depends, however, mainly on morphological evidence, which shows them to be quite distinct from the chondriosomes or

[1] Chapter IX.

[2] *E. g.*, Golgi's silver-method following formol-arsenious acid, or that of Cajal following formol-uranium-nitrate; the method of Kopsch consists simply in prolonged treatment by osmic acid, without other staining. In all these cases the Golgi-elements appear intensely black; but this treatment alone will not always differentiate them from the chondriosomes.

other formed elements, so that they must be considered as specific cell-components *sui generis*.

The Golgi apparatus is of very wide distribution among the cells of higher animals and is known in the Protozoa [1] everywhere showing the same

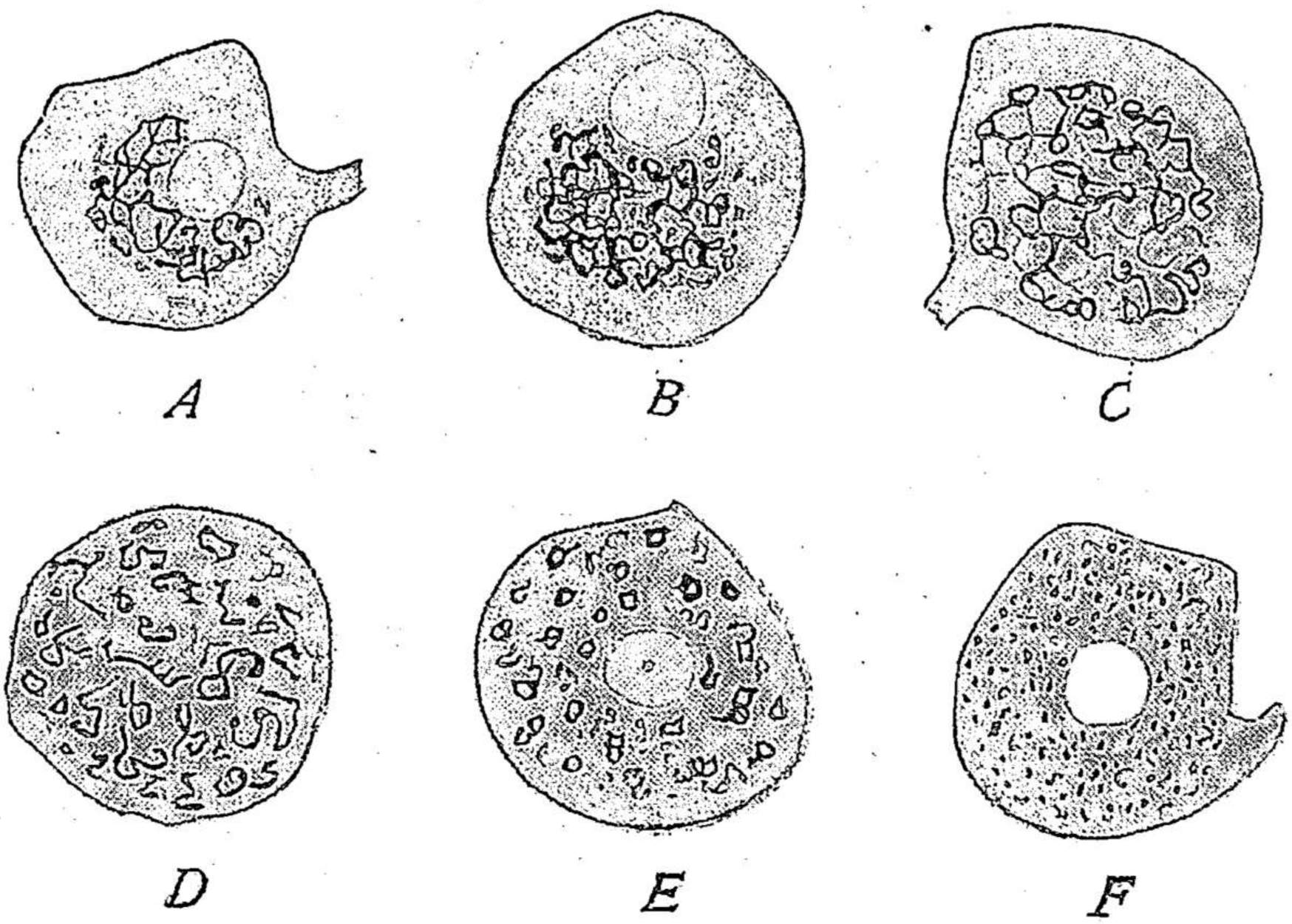

Fig. 20.—Variations of the Golgi-apparatus in nerve-cells, spinal ganglia of young cat (*A*, *C*, *D*; *F*) and rabbit (*B*) (CAJAL).

A–C, reticular type; *D–F*, scattered or diffuse type.

general characters; and there is reason to believe that the same may be true of plant cells though considerable doubt concerning this still exists. It appears in two principal forms, the *localized* and the *diffuse*, which may be converted into one another in changing phases of cell-activity and are therefore to be regarded as merely different phases of the same structural element. In its localized form, as first described by Golgi ('98) in nerve-cells of the spinal ganglia of vertebrates, it commonly gives the appearance of a localized net-like structure, composed of more or less contorted and varicose fibrils, which appear intensely black after silver impregnation or prolonged treatment by osmic acid. This structure Golgi called the *internal reticular apparatus*, a name afterwards widely employed, though in some cases no longer appropriate. In the younger cells and often in the older ones it lies most commonly at one side of the nucleus, but in certain cases may completely surround it (Figs. 20, 21). In the epithelial tissues generally (including the glands) the apparatus typically lies on the side towards the lumen—a fact of much interest in connection with its supposed functions in secretion (p. 52). In the localized form, later observers found

[1] In gregarines, Hirschler ('14), King and Gatenby ('23).

the Golgi-apparatus with many variants, in many other kinds of cells,[1] including the germ-cells (spermatogonia, oöcytes, spermatocytes) though described under other names such as "archoplasmic loops" (Hermann, '91) or "pseudo-chromosomes" forming the "central capsule" (Heidenhain, '00). These structures were shown by Sjövall ('06) and later observers [2] to be identical with the Golgi-apparatus of the tissue-cells and to be quite distinct from the chondriosomes (with which they were formerly confused). It seems to be well established that in many cases the Golgi "net" is built up from originally separate bodies,—lamelliform, rod-like, banana-shaped or the like—and that in such cases they do not lose their identity in the so-called network. These bodies are variously designated as "batonettes," "dictyosomes," or *Golgi-bodies*. They may separate and scatter through the cell to form the diffuse type and again concentrate to form the localized type or "reticular apparatus"; and there is some evidence that the net-like appearance may be an artifact produced by imperfect fixation of the separate bodies.

In its localized phase it commonly surrounds the centrioles and there is some reason to suspect that the so-called "sphere," "idiozome" or "archiplasm-sphere" of the resting cell may be composed of substance that belongs to the Golgi-apparatus (p. 361).[3] This relation to the central bodies was first clearly seen in case of the germ-cells ("pseudo-chromosomes" of Heidenhain, '00) and in epithelial cells of Descemet's membrane ("centrophormium" of Ballowitz, '98, '00) and was later described in many other cases, including the nerve-cells, epithelial cells of various kinds, gland-cells, cartilage-cells, the primordial germ-cells, spermatogonia, spermatocytes and oöcytes.[4] The Golgi-apparatus is not to be confused with the chondriosomes, which likewise may be aggregated about the central bodies but lie more peripherally and often show no definite grouping about the centers (Figs. 22, 149).

The localized type of Golgi-apparatus is connected by many intergradations with the diffuse. Even in the nerve-cells of vertebrates, as shown by Cajal ('08, '14) and other observers the structure shows wide variations, the reticulum often breaking up into separate islands or even into separate rods or granules scattered through the cell (Figs. 20–22). On the other hand, in the nerve-cells of gasteropods (Weigl) and Crustacea (Poluscynski) the apparatus is said to be permanently diffuse, appearing in the form of

[1] For reviews and literature-lists see especially Duesberg ('12, '14, '20), Nussbaum ('13), Pappenheimer ('16), Hirschler ('17, '19), Gatenby ('16, '17, '18, '19), Nassonov ('23).

[2] See especially Weigl ('12), Hirschler, Gatenby (*op. cit.*), Bowen ('20, '21).

[3] See Bowen, '20, '22.

[4] See Sjövall ('06), Barinetti ('12), Pensa ('13), Weigl ('12), Terni ('14), Cajal ('14), Berenberg-Gossler ('13), Hirschler ('18, '19), Duesberg ('20), etc.; also the earlier works of Hermann and Heidenhain.

granules, curved rods (batonettes) plates, or ring-like bodies scattered at random through the cell. Such diffuse forms have now been found in many kinds of cells, either as a permanent condition or one that is incidental to other cell-activities. The most interesting of these cases are found in embryonic cells and during the mitotic division of the tissue-cells. In later stages of development (birds, mammals) as shown by the work of Golgi, Sjövall, Marcora and others,[1] the Golgi-apparatus is of localized and net-

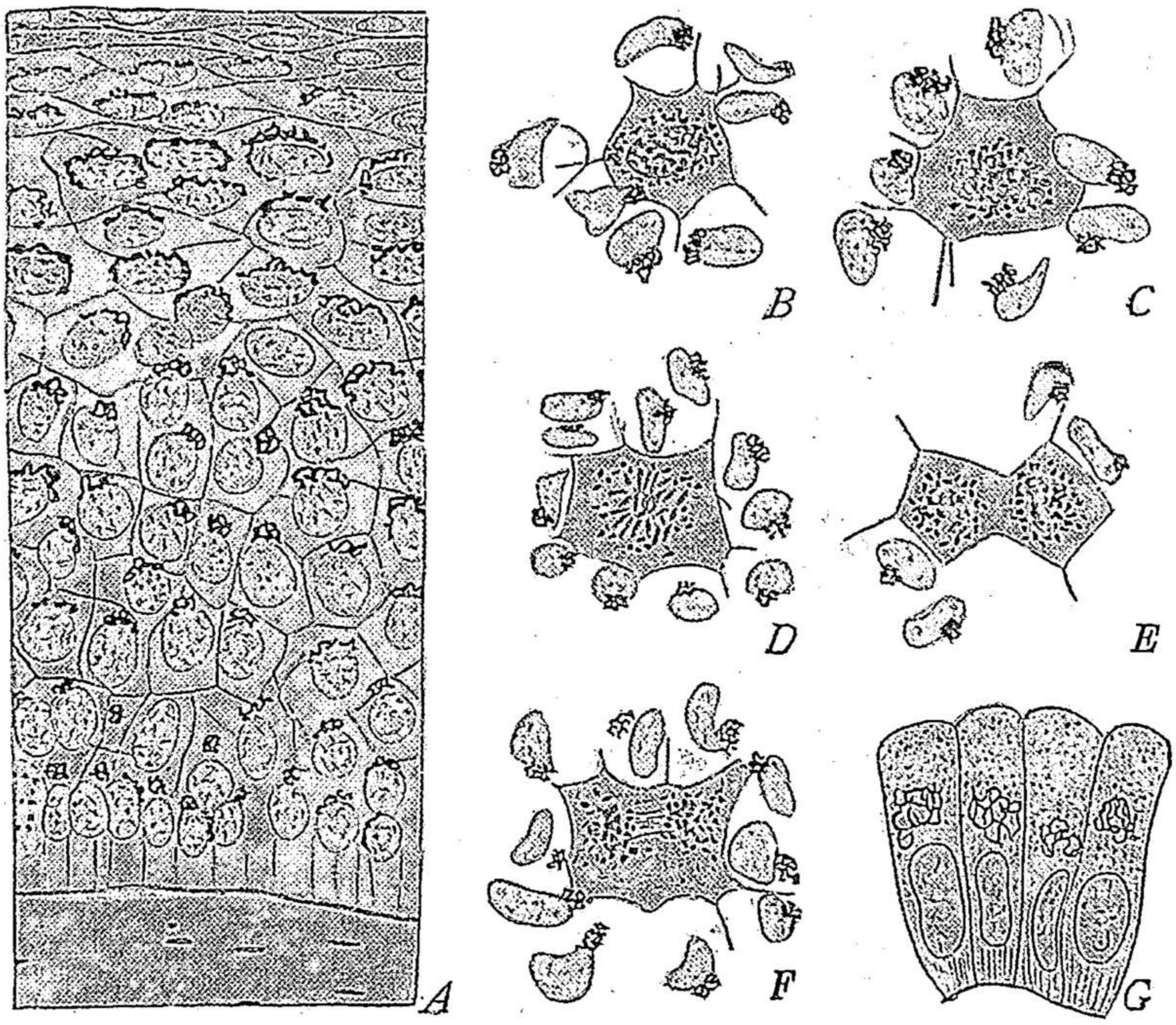

Fig. 21.—Golgi-apparatus and dictyokinesis in epithelial cells, Golgi-method. (*A–F* from DEINEKA; *G*, from BERGEN).

A, vertical section, epidermis of the horse; *B–E*, successive stages of division (dictyokinesis) from Descemet's membrane, new-born cat; *G*, columnar epithelial cells, prostate of dog.

like type; but it was observed by Fañanas ('12) that in early stages of the hen's egg (44 hours and later) it appears in the form of separate, scattered rod-like bodies, which only in later stages of development become aggregated to form a localized net at one side of the nucleus. This is confirmed by the more recent independent work of Hirschler ('18) and Gatenby ('19) on pulmonates (*Lymnæa*). Both observers have found the Golgi-elements in the cleavage-stages and as late as the gastrula in the form of separate, scattered bodies, quite distinct from the chondriosomes in form and staining-reactions (Fig. 347).

In the diffuse type the Golgi-bodies proper are readily distinguishable after a suitable technique by their intense blackening after osmic or silver

[1] Review in Hirschler, '18.

impregnation. In some cases, however, and possibly in all, each of these bodies (batonette, etc.) is accompanied by a small spheroid of clear, non-staining substance to which it is closely applied (hence perhaps its curved form). Gatenby calls this an "archoplasm-sphere," and regards it as the same substance as that of the sphere surrounding the central bodies in the localized type, a view supported especially by the history of the Golgi-elements in the sperm-forming cells (p. 364). There is, however, little ground for calling this material by the vague term "archoplasm," and none for supposing it to have any connection with the substance of the division-figure. Preferably, therefore, it may be called simply the "sphere-substance." Recent studies have prominently raised the possibility that this substance forms an integral part of the Golgi-body, and that the large clear sphere (idiozome, etc.) around which the Golgi-apparatus lies when in the localized form may be built up by the aggregation of the smaller spheres accompanying the scattered Golgi-bodies of the diffuse type (p. 361).[1]

These facts seem to show that the localized form is to be regarded as a secondary condition. Additional ground for this conclusion is offered by the history of the Golgi-bodies in the sperm-forming cells, and also by their behavior during mitosis (p. 165).

Concerning the functional significance of the Golgi-elements even less is known than in case of the chondriosomes. The important fact has recently been made clear that they play an important part in the formation of the acrosome during spermatogenesis [2] and perhaps also contribute to that of the middle-piece. The evidence that the Golgi-apparatus may be concerned in the processes of secretion will be considered later (p. 715).

A considerable group of observers, headed by Holmgren ('99, '00 and later), have considered the Golgi-apparatus in its localized or net-like form as a system of intracellular canals filled with fluid which after coagulation and impregnation with silver or osmium appear as solid filaments.[3] Thus arose the term "canalicular system," first applied to the Golgi-apparatus by Holmgren and adopted by many later writers as synonymous with the latter term. Holmgren concluded further that this system arises from ingrowths of the surrounding cells ("trophocytes") which become vacuolated and are finally transformed into canaliculi, forming a hollow network that still communicates with the exterior. Owing to the supposed trophic functions of

[1] See Gatenby ('19), Ludford and Gatenby ('21), Bowen ('20, '22).

[2] *Cf.* p. 381. See Sjövall ('06), Perroncito ('10), Weigl ('12), Cataneo ('14) and especially Hirschler ('19), Gatenby ('17–'19), Bowen ('20, '22), Gatenby and Woodger ('21), Ludford and Gatenby ('21).

[3] Duesberg ('12, '14) gives a valuable review and discussion of this problem, with literature-lists. See also Cajal ('14) and Pappenheimer ('16).

these ingrowths the Golgi-net was designated as a "trophospongium." The terms "Golgi-apparatus," "canalicular system" and "trophospongium" were thus employed for a time as synonyms for the same structure even by observers who differed more or less concerning its nature and

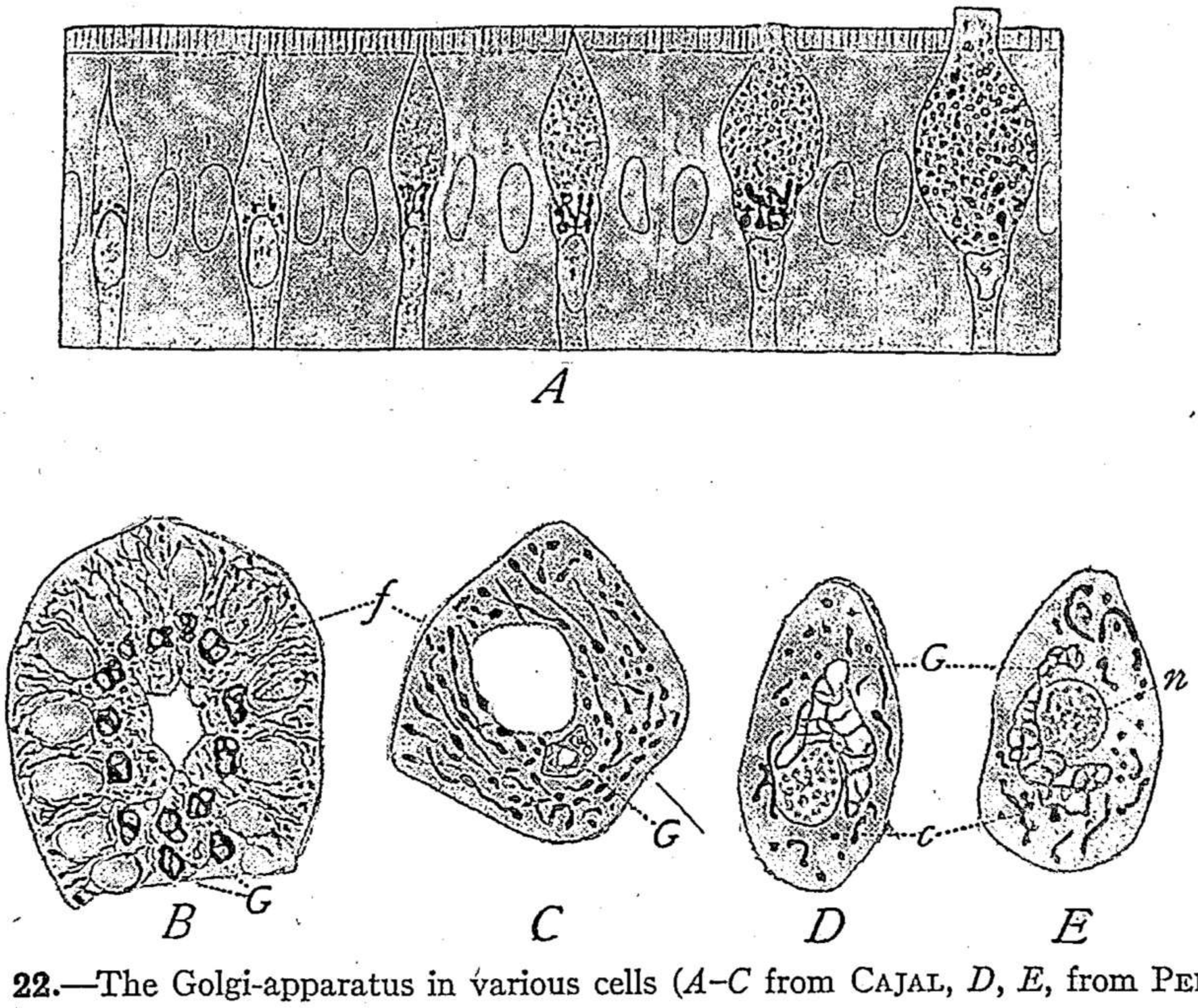

Fig. 22.—The Golgi-apparatus in various cells (*A–C* from CAJAL, *D*, *E*, from PENSA).

A (slightly schematized) progressive functional changes in goblet-cells from the intestinal epithelium of mammal; *B*, pancreas-cells, showing both Golgi-apparatus (*G*) and chondrioconts (*f*); *C*, the same more enlarged; *D*, *E*, cartilage cells, showing Golgi-reticulum (*G*) and chondriosomes (*c*).

origin. It now seems probable, as emphasized especially by Duesberg, that two quite distinct structures were confused in this usage, one the Golgi-apparatus proper, the other a system of trabeculæ or fibrils formed as ingrowths from the surrounding cells. Such ingrowths, forming a true "trophospongium" seem to have been clearly demonstrated in a number of cases, in particular by Nussbaum ('13) and by Ross ('15) in nerve-cells; but these and other observers are now agreed that this structure is quite distinct from the Golgi-apparatus. Some competent observers (Cajal, Bensley, Cowdry) have adopted Holmgren's conclusion that the Golgi-net represents the coagulated, lipoid-containing contents of a system of intracellular canaliculi, but have found no evidence of its connection with the exterior or of its derivation from "trophocytes" or other surrounding cells.

An external origin of the Golgi-apparatus seems to be quite excluded by its behavior during mitosis and also by the behavior of the scattered, or diffuse Golgi-bodies in the early embryonic cells. In such cases the Golgi-bodies can at most be regarded as vacuoles or their coagulated contents,

which at no time have any connection with the exterior. It is, however, difficult thus to regard the scattered Golgi-bodies as vacuoles (or their contents) in view of their shape and other peculiarities; and it seems more natural to consider them as definite bodies, solid or semi-solid, that have no connection with the trophocyte-ingrowths observed by Holmgren and Nussbaum.[1]

7. Vacuoles

Vacuoles are found in many kinds of cells, conspicuously developed especially in the tissue-cells of higher plants generally and in many of the Protista. They are in general spheroidal cavities containing a watery liquid, and probably always bounded by a delicate protoplasmic limiting film comparable with the external plasma-membrane (p. 55). Certain forms of vacuoles (in the swarm-spores of lower plants, and in many Protists) possess the power of rhythmical pulsation and play an important part in excretion. In some cases vacuoles have the power of division, and for this and other reasons have been considered as products of special forms of plastids (*tonoplasts* of De Vries). Some authors have gone so far as to conclude that vacuoles (or tonoplasts) arise only by the division of preëxisting vacuoles,[2] but it seems unlikely that this is of general validity. In many Protozoa, for example, solid food is digested in the interior of vacuoles which may be seen to form *de novo* during the ingestion of food. The power of division, therefore, is probably confined to certain special forms of vacuoles.

The prominence of vacuoles in the cells of higher plants as contrasted with those of animals has earlier been indicated. In higher animals, generally, vacuoles are either wanting or inconspicuous; in many of the Protista (ciliates, rhizopods), on the other hand, they are often developed to such an extent as to give the whole protoplasm a foam-like or pseudo-alveolar structure (p. 72). If, as above suggested, the canalicular system of Holmgren may be comparable to a vacuolar system, the latter would be of more general occurrence in lower animals than has generally been supposed.

8. The Cell-membrane or Wall

All kinds of cells are probably limited externally by some kind of membrane, though this is not always evident to the eye. Animal cells are in general characterized by a relatively slight development of the membrane,

[1] For further facts concerning the Golgi-apparatus see p. 714. Bensley ('10) has concluded that in plant-cells the "canalicular apparatus" is represented by the vacuoles, at first forming an intricate closed network of fine canals, quite comparable to the Golgi-apparatus of animal cells. This view, which has been supported by Guilliermond, recalls a suggestion of Cajal's that the canalicular system (Golgi-apparatus) in the cells of higher animals may be comparable to the contractile vacuole with its associated drainage-canals in ciliates.

[2] DeVries, '85, Went, '88.

which is often so thin and delicate that the cells appear to the eye naked, and in many cases were formerly so described. The plant-cell, on the other hand, commonly shows sharply marked and often very thick walls, to which circumstance indeed the cell owes its name (p. 22). This difference is, however, a superficial one arising from the existence of two widely different types of cell-membrane, one of which is common to all types of cells while the other varies widely in the extent of its development and is often absent. There are, respectively, the plasma-membrane [1] and the cell-wall proper, sometimes referred to as the "true membrane." The first of these belongs to the protoplast proper and forms part of the cytoplasm. The second lies outside the cytosome, in some cases separated from it by a considerable space, and is generally regarded as a non-protoplasmic or metaplasmic product of the cytoplasm, though in many cases it possesses, like protoplasmic structures, the power of growth by intussusception. By many writers the term "cell-wall" is reserved exclusively for membranes of this type.

As now commonly employed the term plasma-membrane designates a thin peripheral surface-film or limiting layer of cytoplasm, differing in physical consistency from the underlying substance, but often indistinguishable cytologically. Its presence is demonstrated by experiments on plasmolysis, the penetration of various dyes and the like, which prove this layer to have the properties of a semi-permeable membrane and one which plays a most important part in regulating the exchanges of the cell with its environment, and in the stimulation of the protoplasmic activities.[2] Secondly the existence of the plasma-membrane is more directly established by the so-called "micro-dissection" or "micro-vivisection" method due to Barber and Kite and developed particularly by Chambers.[3] Experiments by this method prove that the plasma-membrane or surface-film of protoplasm is of firm or even tough consistency, in some cases highly elastic, and offers considerable resistance to mechanical injury. A similar membrane is formed about the protoplasmic vacuoles, and also about naked masses of protoplasm produced by cutting, tearing, or shaking cells to pieces.

Modern studies on the plasma-membrane indicate that it is comparable

[1] Also called ectoplasm, ectoplast, Hautschicht, Plasmahaut, etc. See Glossary.

[2] See R. S. Lillie, '09, '13, '14, '20, '24.

[3] See Barber ('14), Kite ('12, '13), Chambers ('17, '19, '24), Seifriz ('18). These operations are performed on living cells suspended in hanging drops (of sea-water, blood-serum or other appropriate normal fluid) by means of the "micro-dissection-needle" devised by Barber. Such needles are made by drawing out small glass rods to an extremely fine point and can be used to puncture, cut, tear or displace the living cell-substance under high powers of the microscope. Their use by the observers mentioned has afforded important data concerning the physical nature of the cell-substance. Accurate control of these operations is made possible by a number of simple mechanical appliances readily attached to the stage of the microscope. See Barber ('14) and, for fuller accounts, Chambers ('15, '17).

physically to the surface-layer or film that tends to form at the free surfaces (or the interfaces) of non-living colloidal systems; and on this basis many interesting experiments and hypotheses have been put forward in attempts to show how the osmotic phenomena of the cell are controlled by changes in the permeability of this layer [1] and how this may play a part in cell-division and fertilization (pp. 191, 410). It has been plausibly argued by Overton, Loeb and others that the plasma-membrane owes some of its characteristic properties to its richness in lipoid substances (hence the effect of fat-solvents in artificial parthenogenesis, etc.). Others have urged the emulsoid nature of the plasma-membrane and the variations of viscosity owing to the inversion of phases (Clowes), or the degree of dispersion of the suspended phase; [2] but the questions raised by these inquiries lie outside the scope of this work.

The outer or true membrane, often wholly wanting, shows a very wide diversity of development, chemical composition and other characters. It is typically exemplified by the cellulose walls of plant-cells or the cuticular membranes often formed on the free surfaces of epithelial cells in animals. In free (solitary) cells the true membrane forms a distinct surrounding envelope. In the tissues the membranes form intercellular partitions or walls between the cell-bodies, often consisting of several layers. The cell-bodies may become widely separated by continued growth of the intercellular substance, so that in extreme cases (*e. g.*, in cartilage) they lie scattered in a non-living "matrix."

The structure of the wall may be studied to greatest advantage in the higher plants, where its origin and nature have occupied the close attention of many eminent botanists. Some of these, impressed with its growth by intussusception, have considered it to be "living," or at least to arise by a direct transformation of the peripheral protoplasm (Wiesner, Molisch, Haberlandt and at first Strasburger, etc.). At present the earlier view of Mohl and Nägeli is generally accepted that it is a secretion-product of the protoplasm, either at the periphery of the cell or in the interior of the cell-plate (p. 159). In the higher plants generally it usually shows a central layer (the middle lamella or primary wall) the presence of which is traceable to the method of cleavage by cell-plate formation. To this layer, composed of pectose (a carbohydrate) succeed on either side "secondary" and often "tertiary" layers, laid down upon the middle lamella after its formation and largely composed of cellulose. These layers, as they grow older, often undergo a variety of chemical and physical changes, including the deposit in them of other organic or inorganic substances, and a great

[1] See for instance Bancroft ('13, '14), Clowes ('16a, '16b), Loeb ('13), R. S. Lillie (*op. cit.*).
[2] Lloyd ('16), Spaeth ('16), Free ('18). See Sharp ('21).

variety of sculpturing, pitting and the like on their surfaces. In animal cells much less is known of the true walls, owing to their greater delicacy and often their lack of visible structure. Many of them (including the intercellular substances generally) are nitrogeneous bodies, such as keratin or chitin; but as in the case of plants they sometimes become impregnated with inorganic deposits, such as silica, lime-salts, etc. Owing to their different mode of formation (*i. e.*, by secretion from the external surface instead of by cell-plate formation) animal membranes do not show a middle lamella; and though in exceptional cases they may become greatly thickened, they do not in general show the distinction between primary, secondary and tertiary walls.

III. PROTOPLASM. ITS COMPOSITION AND STRUCTURE

The structure of protoplasm has always offered a problem of primary interest to students of the cell; for it seemed that we might expect here to gain some insight into the mechanism of the protoplasmic activities. This was early urged by the eminent physiologist Brücke (1861), who argued that the activities of cells demand for their explanation the assumption of a fundamental organization or architecture of protoplasm as distinguished from its merely chemical or physical properties.[1] It seemed a reasonable hope that at least some of the features of such an organization might appear in a visible structure of the protoplasm; and this has led to prolonged cytological study of the problem. If this hope has thus far had a rather meager fulfillment, the problem still retains a fundamental interest and attempts to solve it have played a very important part in the advancement of our actual knowledge of the cell. It is necessary to approach the subject by some preliminary discussion of our use of terms.

1. Terminology

Max Schultze, Kühne, DeBary, Hanstein and other earlier observers of protoplasm (cytoplasm) described it as a clear substance, having the general properties of a viscid liquid, and containing granules. They thus recognized in rudimentary fashion the fact that protoplasm is not a homogeneous or single substance but a mixture of different components; and this conclusion has constantly gained in weight with the advance of later researches both on the chemistry of the cell-substance and on the visible forms which it may assume. Many of the visible cell-components (such as various forms of granules and fibrillæ) differ markedly in different kinds of cells and often seem to be of secondary origin, arising in the course of differentiation, or coming and going with different phases of the cell-activities. Some of these

[1] See quotation at p. 632.

secondary elements, such as starch-grains or fat-drops, behave like inert and lifeless bodies; and this fact led to the conception of a fundamental living protoplasm as distinguished from its "non-living" products. Lionel Beale (1861) drew a sharp distinction between the primary, "formative," "germinal" or "living" matter of the cell (afterwards called *bioplasm*), and the "formed material," maintaining that "the changes which more especially distinguish living structures from lifeless matter take place in the substance that I have termed *germinal matter*, and in this alone." [1] Van Beneden (1870) distinguished, in case of the animal egg, an active "protoplasm," and a passive "deutoplasm," consisting of storage products in the form of yolk. Hanstein (1880) in like manner contrasted the active, living protoplasm with the passive or lifeless *metaplasm* to which it may secondarily give rise; while Sachs (1892, 1895) distinguished the living *energid* (active protoplasm and nucleus) from the passive *energid-products* (metaplasm). Already in Beale's work, however, appears a further distinction between "formed material" and "secondary deposits," the latter being considered as wholly passive products of the formed material: as examples of such deposits he gives the starch-grains and fat-drops. This distinction, though not very logically carried out, foreshadowed later attempts to find a more adequate classification of the cell-components. For instance Kupffer (1896) recognized in addition to the active protoplasm of the energid two types of formed material or energid-products, the active or *dynamoplastic* (such as *myofibrillæ* or *neurofibrillæ*) and the passive or *paraplastic* (metaplasmic of Hanstein). Arthur Meyer similarly grouped the cell-components into three classes, as follows: [2]

a. Protoplasmatic: comprising the primary and most active elements, represented by the undifferentiated or fundamental cell-substance, the nucleus, the plastids, and perhaps the centrioles, all of which possess the powers of growth and self-perpetuation and arise by division of preëxisting elements of the same kind.

b. Alloplasmatic: (=dynamoplastic of Kupffer), assumed to be secondary products of differentiation of the protoplasm, and not self-perpetuating, but performing active functions. Examples of such structures were considered to be cilia, flagella, myofibrillæ, neurofibrillæ, astral rays and spindle-fibers. Meyer included here also the "tonoplasts" of DeVries, or vacuolar walls.

c. Ergastic: (=paraplastic, metaplastic) relatively passive secondary products of differentiation, in the form of "inclusions" (starch-grains, fat-drops, etc.) or external secretions (intercellular substances), all of which are often spoken of as "lifeless."

[1] Q. J., 1862, p. 80.

[2] See Meyer, '96, '12. Certain modifications of Meyer's categories have been here added.

No attempt is here made to identify a living "protoplasm" as such or to distinguish between "living" and "non-living" cell-components. Life is treated as a property of the cell-system as a whole, the components of that system differing only in the degree and manner of their activity. In this respect Meyer's views are quite in agreement with those earlier expressed by Hanstein, Flemming,[1] Kölliker, O. Hertwig and other leading students of the subject, and the same view of the matter has been adopted by many modern physiologists and biochemists who regard the cell as essentially a complex colloidal system.[2]

Logically, no doubt, this is correct; and from a purely physiological point of view is perhaps the only possible mode of treatment. The cytologist, however, finds it convenient to distinguish, as did Beale, between a primary undifferentiated substance that is common to all kinds of cells and the formed components that may appear within it. The former substance is probably to be identified with the *hyaloplasm* or clear ground-substance in which the differentiated protoplasmic elements are suspended. Many of the latter have been spoken of, even by recent writers, as protoplasmic "inclusions"—obviously an inappropriate term in view of the fact that they may play an essential part in the cell-activities. We shall therefore call them *formed bodies*[3] without attempting to distinguish at this point between "living" and "non-living" cell-components, or between "formed material" and "secondary deposits."

2. Chemical and Physical Properties of Protoplasm

Chemically considered, protoplasm is a complex mixture, comprising especially *proteins* and their many derivatives; the *lipoids* or fatty bodies; *carbohydrates;* and *inorganic salts*, together with a large amount of associated water. In these respects the protoplasm of animals and plants shows a general similarity, though the relative proportions of the protoplasmic components varies widely in different organisms and even in different physiological states of the same species. The earlier work on protoplasm emphasized the protoplasmic resemblances between plants and animals and laid especial weight on the importance of the proteins in both.

[1] "The moment we enter upon the question as to whether this substance or that is still to be called protoplasm or is no longer such, we are treading on uncertain ground, simply for the reason that no man can definitely say *what protoplasm is*. . . . That which lives is, in my view, the entire body of the cell" (Flemming, '82, pp. 78, 81).

[2] *Cf.* pp. 633, 635. "We cannot, without gross misuse of terms, speak of the cell life as being associated with any particular type of molecule. Its life is the expression of a particular dynamic equilibrium which obtains in a polyphasic system. Certain of the phases may be separated . . . but life, as we instructively define it, is a property of the cell as a whole, because it depends upon the organization of processes, upon the equilibrium displayed by the totality of the coexisting phases." (F. G. Hopkins, '13, p. 213).

[3] This is taken from Beale, but used in a somewhat broader sense.

More recent studies have shown, however, that marked chemical differences in this respect exist between the two groups, at least in higher forms, the carbohydrates being much more prominent in the protoplasm of plants, while the proteins and lipoids predominate in that of animals.[1]

Physically, protoplasm displays the properties of a complex colloidal system and commonly behaves as a viscous liquid. As such a liquid protoplasm was described by all the earlier investigators such as Dujardin, Schultze, Kühne and DeBary. These observers, and their followers, using relatively low powers of the microscope, described living protoplasm as consisting of a clear, homogeneous, viscid ground-substance or *hyaloplasm* containing suspended granules or *microsomes*[2] of various sizes, and often also vacuoles filled with a watery liquid. Bütschli (1878), working largely on living Protozoa, where the vacuoles are often very small and closely crowded, suggested that protoplasm has a foam-like or "alveolar" structure, similar to that of an emulsion; and he afterwards ('92) developed this conception into a general theory of protoplasmic structure, which, as will later be seen, is quite in harmony with more modern views concerning the colloidal properties of protoplasm.

The viscous liquid nature of protoplasm is patent in cells which display flowing movements of the living protoplasm, as in cyclosis or in the formation of pseudopodia. Free cells, when in a state of rest, tend towards the spheroidal form, while actively irregular cells such as *Amœba* or leucocytes generally become spheroidal upon electric shock. Living fragments of protoplasm, produced by shaking or cutting cells to pieces, generally round up to a spheroidal shape. Watery vacuoles in protoplasm are typically spheroidal; and they often move freely through the protoplasmic substance, as may also the cell-nucleus, plastids, granules, yolk-spheres and other formed bodies. Cells originally separate may completely fuse to form a single body, a process which occurs naturally in the conjugation of gametes and may be artificially induced in case of the eggs of sea-urchins and other animals (p. 972). There is strong evidence that during cell division the astral rays, possibly even the spindle-fibers, are lines of protoplasmic flow; while vortical and other movements of the peripheral protoplasm also may be observed at this time (pp. 192, 198).

Numerous researches in recent years have, however, proved that the protoplasmic viscosity varies widely in different kinds of cells and even in different physiological states of the same cell, and that it may sometimes reach a point at which the protoplasm passes over temporarily into a jelly-like or semi-solid condition. Such solidifications and liquefactions may occur

[1] See MacDougal, '20.

[2] These terms are due to Hanstein, 1880.

in living protoplasm as reversible processes which play an important part in the life of the cell (p. 197).

In its more liquid condition protoplasm (or rather the apparently homogeneous ground-substance or *hyaloplasm*) shows many of the properties of a watery colloidal solution, consisting of a continuous, watery, more liquid substance in which are suspended a multitude of very minute and often ultra-microscopic particles or droplets, electrically charged and in some cases (as demonstrated by the ultra-microscope) in active Brownian movement.[1] This movement is of course only possible in a liquid medium, and is much retarded, or ceases, when the viscosity of the medium increases beyond a certain point. In most cases the movement is not shown by the *visible* granules of living protoplasm, thus indicating a considerable degree of viscosity in the protoplasmic substance; but active Brownian movements of the visible granules are seen as soon as the protoplasm liquefies after death. The occurrence of such movements in living protoplasm seems, it is true, to be authenticated in a few cases; [2] but, as was long since pointed out by Flemming, who observed the dance of minute fat-drops in living cartilage-cells ('82, p. 50), it is not easy to exclude the possibility that such granules may lie in watery vacuoles in the protoplasm or that a sub-mortem liquefaction is in progress. In the cytoplasm of the sea-urchin egg during its more liquid phase no Brownian movement of the microsomes is seen in the living object; [3] but when the protoplasm is killed by crushing or tearing active Brownian movements appear, while the alveolar spheres or macrosomes swell and disappear (Chambers).[4] On the other hand, the ultra-microscope (p. 33) reveals the existence in the ground-substance or hyaloplasm of living cells particles that lie beyond the reach of the ordinary microscope, which often are seen to be in active Brownian movement.[5] According to Gaidukov the movements of these particles cease upon death of the cell, which he ascribes to a post-mortem rigor or coagulation; and he also showed that in the protoplasm of *Vallisneria* an active Brownian movement of the particles is seen under the ultra-microscope (dark ground

[1] This movement, so called after its discoverer, Robert Brown (1828), is a rapid trembling movement of minute particles suspended in a liquid medium, readily seen in an aqueous suspension of finely powdered gamboge, carmine or lampblack. Its cause is now generally referred to bombardments of the suspended particles or granules by the molecules of the liquid in which they are suspended. Other things equal, the amplitude of the vibrations is inversely proportional to the size of the granules, and the smaller ones may have a slow and irregular, but very considerable, movement of translation. For an account of this subject see Bayliss, *Principles of General Physiology* ('15), and the work of Perrin ('10) there cited.

[2] See F. R. Lillie, '06.

[3] Wilson, '99, Chambers, '17.

[4] More or less complete dissolution of the macrosomes also often takes place on fixation of the cytoplasm by certain agents such as acetic or picric acid; hence the difficulty of proper fixation of alveolar protoplasm by many reagents containing a high percentage of such substances.

[5] See Gaidukov, '10, Mainesco, '12, Price, '14, Bayliss, '20, etc.

illumination) so long as the protoplasm is moving. When the latter movement ceases locally the Brownian movements likewise cease, but reappear just before the protoplasmic movement is resumed. This means, of course, that the viscosity of the protoplasm increases during rest and decreases during movement. Somewhat similarly, Bayliss ('20) shows that in the ectoplasm of a living *Amœba*, so long as the protoplasm is moving, the minute particles made visible by dark ground illumination show an active Brownian movement, but this ceases at once when the protoplasm is electrically stimulated, to be resumed when the stimulation ceases and the protoplasmic flow reappears. With a stronger stimulus the protoplasm is killed and the Brownian movements cease at once, not to be renewed until the protoplasm liquefies during *post-mortem* changes. These changes, manifestly, demonstrate the variations in degree of viscosity associated with protoplasmic movement; and as Bayliss points out, they are quite analogous to the cessation and reappearance of the Brownian movements of minute suspended solid particles in a solution of gelatin alternately cooled and warmed.

Wide variations in the physical consistency of the protoplasmic substance have been demonstrated by micro-dissection studies on living cells and also by the use of the centrifuge.[1] According to Kite the protoplasm of epithelial cells or of nerve-cells shows such a degree of rigidity that it may be cut to pieces which undergo little or no change of form. The living substance of the muscle-cell is also fairly solid but more viscous and highly elastic, so that it may be drawn out into long threads which when released almost regain their previous shape. On the other hand, in the "resting state" or interkinesis of many cells—such as egg-cells (echinoderms, nemertines), sperm-forming cells, or Protozoa generally—the protoplasm has the properties of a viscid liquid bounded by a plasma-membrane that is of much more solid consistency. During the mitotic activities of these cells Heilbrunn and Chambers have shown that a large part of the protoplasm temporarily undergoes a process of solidification or gelation, returning to the more liquid state at the close of division (p. 197). The variations in viscosity displayed by protoplasm are evidently comparable with those seen in emulsoid colloidal substances, and when in its more solid condition protoplasm may be analogous to the "gel" or solidified state of colloidal substances generally. The problems here encountered are, however, of great complexity. The larger particles suspended in the hyaloplasm undoubtedly vary widely in physical consistency, being in many cases more solid "granules," in other cases more liquid "drops";

[1] Barber, '11, '14; Kite, '12, '13; Kite and Chambers. '12; Chambers, '14, '15, '17, '18, etc.; Heilbrunn, '15, '17; Seifriz, '18, '20.

but obviously it is difficult to draw any definite boundary line between these two extremes. The hyaloplasm or ground-substance in which these bodies lie is often of more liquid nature and has generally been held to be comparable to an "emulsoid" [1] containing ultra-microscopical suspended drops (in contradistinction to a "suspensoid" in which the dispersed particles are solid); but the grounds for such a conclusion seem inadequate. In any case, when speaking of the protoplasmic viscosity in general, we refer to the properties of the whole protoplasmic complex rather than to any of its particular components.

The behavior of the cell-substance as a whole may be greatly affected by the nature and amount of its formed components since these may be scanty or abundant, and of all degrees of viscosity from liquid watery drops to solid granules or even crystals. The nature of the inorganic salts present also has an important effect on the protoplasmic consistency.[2]

3. Fibrillar Theories of Protoplasm

The results of the earliest accurate cytological studies of protoplasm are embodied in the fibrillar theories, which sought the fundamental structure in delicate fibrillæ, either separate or forming a connected network, traversing a homogeneous ground-substance (Fig. 23). These theories are especially associated with the names of Heitzmann, Klein, Leydig, Flemming, Carnoy, Van Beneden, Retzius and, at a later period, Boveri, Watasé, Heidenhain and Ballowitz. Most of these writers regarded the fibrillæ as fundamental structural components of the cell-substance, ascribing to them a leading rôle in the protoplasmic activities; and even Flemming, who clearly recognized that life belongs to the cell-system as a whole (p. 59), considered it a probable hypothesis "that the essential energies on which life depends have their seat in the fibrillæ." [3]

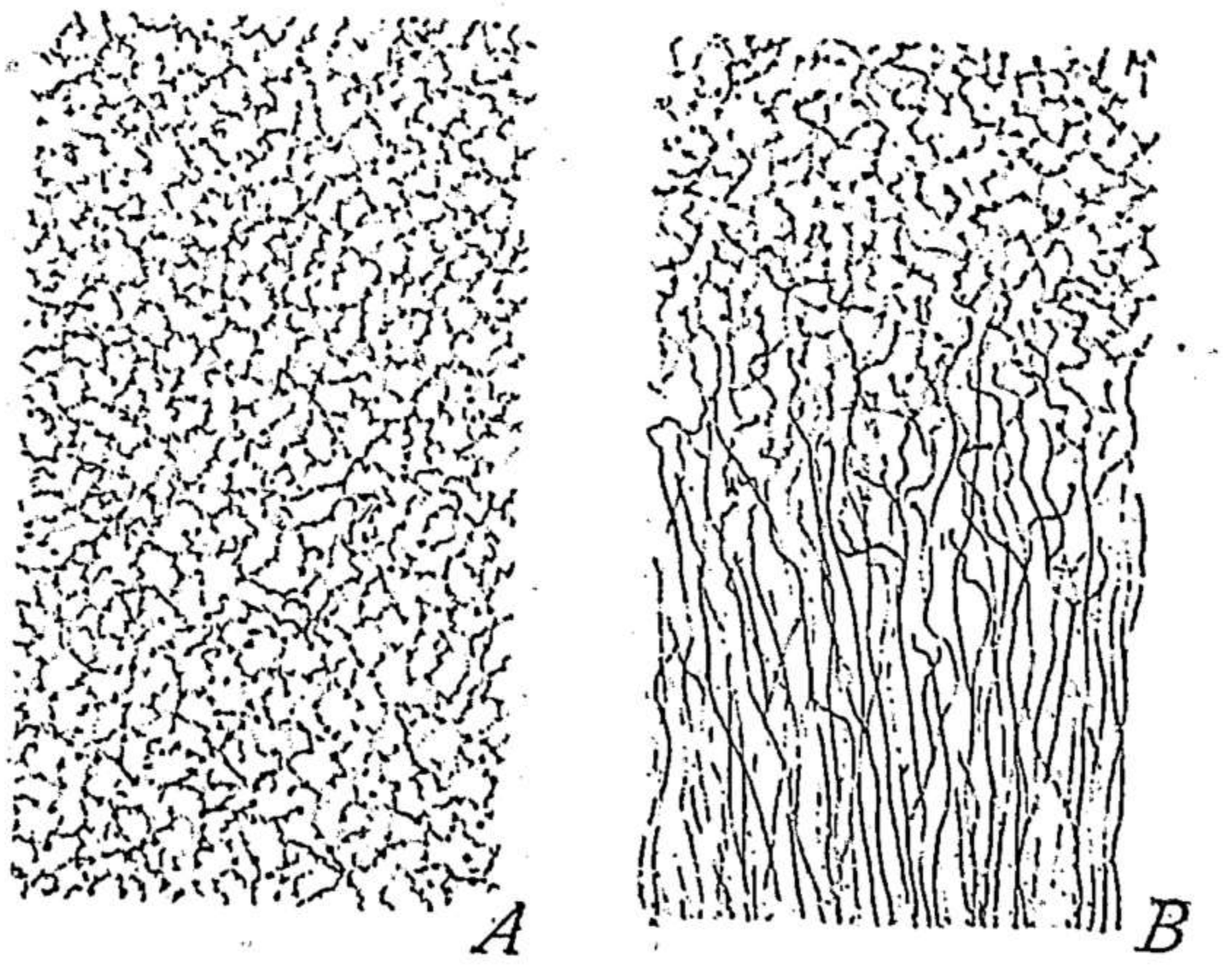

Fig. 23.—Reticular structure (probably a coagulation-product) in fixed and stained section of early blastomere of segmenting fish-egg (*Coregonus*). In *B* are shown the outer extremities of astral rays from a large aster lying below.

The early fibrillar theories of protoplasm arose through a shifting of the center of interest from studies on living protoplasm to those on fixed and stained material, a change largely due to the rapid development of

[1] See Kite, '13. [2] Clowes (16), etc. See also Seifriz ('21). [3] '82, p. 80.

cytological technique between 1870 and 1890. To this development, undeniably, we owe the discovery and elucidation of some of the most fundamental phenomena of the cell; but we now see that the results were in some respects misleading, and that some of the ablest observers fell into error because of a failure to distinguish between the structure of *living* protoplasm and that of the artificial coagula presented by sections. This applies particularly to the net-like formations; for, although these may perhaps really exist in some forms of living protoplasm (p. 40) they may also readily be produced in homogeneous colloidal solutions, *e. g.*, of albumin and gelatin, when coagulated by the ordinary cytological fixing solutions (p. 65).[1] As observations in this field multiplied, fibrillar conceptions of protoplasm grouped themselves into two general views, which may be designated as the *reticular* and the *filar* theories of protoplasm. By advocates of the reticular theory, such as Heitzmann, Kupffer ('75), Leydig ('67), Klein ('78), Van Beneden ('83), Carnoy ('85) and their followers, the fibrillæ were assumed to form a fine, continuous network or reticulum, extending throughout the cell and even from cell to cell (Heitzmann). On the other hand, Flemming ('82), and later advocates of the filar theory, such as Heidenhain and Ballowitz, believed the fibrillæ to be in general unbranched and discontinuous. The correctness of this view, up to a certain point, was from the first made evident by Flemming's demonstration that such separate fibrillæ may readily be seen in the living cells of cartilage and some other tissues (Fig. 9); and this has been fully confirmed by the later observations of Meves ('10), Fauré-Fremiet, ('10) Lewis and Robertson ('16), and others.

Under the influence of these views arose special terminologies differing more or less with different observers. The fibrillar threadwork was variously designated as the *protoplasm* (Kupffer), *spongioplasm* (Leydig), *reticulum* (Carnoy, Van Beneden), *filar substance* or *mitome* (Flemming); the clear intermediate substance as the *paraplasm* (Kupffer), *hyaloplasm* (Leydig), *cell-sap* or *enchylema* (Carnoy), *interfilar substance* or *paramitome* (Flemming). All these observers were substantially agreed that minute granules or *microsomes* are often scattered along the threads or collected at the nodes of the network. By Heitzmann (1873) the reticulum was believed to be continuous throughout the body, the cells being only local areas within it, and the nuclei local areas of concentration within the cells. The whole body was thus conceived as a continuous protoplasmic unit. Muscle-fibers, nerve-fibers, and the like were conceived to be special local modifications of the general network. A similar conception was by later writers applied

[1] Among the earliest observers to describe fibrillar structures in protoplasm were Frommann ('65, '67, '75), Heitzmann ('73), Arnold ('65), and Kupffer ('75).

to the astral and spindle formations in mitotic cell-division, which were assumed to arise by local regrouping of the preëxisting threadwork about the centrioles or division-centers (Klein, Van Beneden, Heidenhain); and by assuming these fibrillæ to be contractile or in a state of elastic tension attempts were made to offer a mechanical explanation of the division of both the nucleus and cell-body (p. 178).

4. Coagulation Phenomena

In the meantime, doubts arose in regard to the reticular and other fibrillar formations in protoplasm. From the first, Bütschli's studies on living protoplasm had led him to a different conception of the protoplasmic framework, while Flemming ('82) and later Berthold ('86), Schwarz ('87) Bütschli ('92) and A. Fischer ('94) had called attention to the danger of confusing

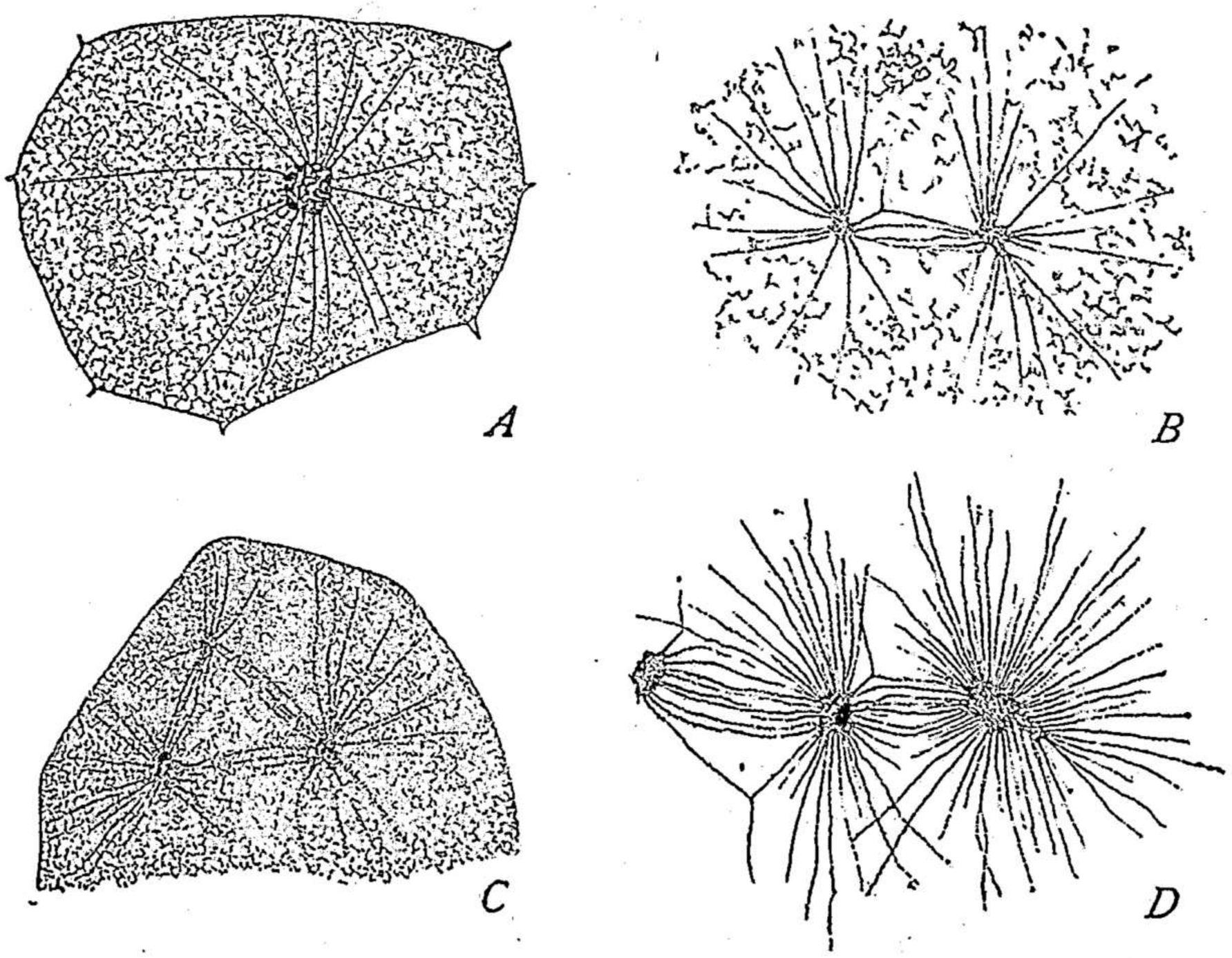

Fig. 24.—Coagulation-artifacts imitating cell-structures (FISCHER).
A, dead pith-cell impregnated with 5% albumin and 2.5% hæmoglobin and fixed in 1% osmic acid; *B*, 2% serum-albumin fixed in Flemming's fluid; *C*, 5% albumose solution in 5% gelatin, fixed in 1% osmic acid and 1% acetic; *D*, 2.5% albumose solution fixed in 1% osmic acid.

coagulation-artifacts with the normal structural elements. In 1899 the importance of this subject was brought to general attention particularly by the work of Hardy and of A. Fischer; and one can only agree with Hardy's opinion that it is "one of the most remarkable facts in the history of biological science that the urgency and priority of this question should have appealed to so few minds." [1] The studies of these observers proved that

[1] '99, p. 160.

the artificial coagulation of homogeneous solutions of albumose, gelatin, egg-albumin, peptone and similar substances may give rise to beautiful net-like or alveolar formations and even to close *simulacra* of astral rays and spindle-formations. Fischer, after impregnating dead and empty pith-cells with albumose solution, and fixing, sectioning and staining by the most approved cytological methods, obtained startling imitations of normal cells, showing fine protoplasmic networks, while about solid particles (such as the dead remains of nuclei) the fibrillæ assume an aster-like disposition and between them give rise to spindles (Fig. 24). The amphiaster was likewise imitated by Bütschli ('98) in gelatin solution containing air-bubbles, suddenly coagulated while hot, astral rays being formed about the bubbles and spindle-like formations between them. Hardy in like manner showed that a film of albumin, weighted in the middle with a drop of mercury and coagulated, shows a striking, aster-like figure of fibrillæ radiating from the position of the weight.

It is not possible here to enter far into the intricacies of the mechanism of coagulation-phenomena. The studies of Fischer and of Hardy proved that when colloidal solutions like white of egg or gelatin are coagulated (an irreversible process) by fixing agents, such as sublimate, osmic vapor, or alcohol, there is a separation of more solid substances from the more liquid, in such a manner as to form a comparatively coarse framework readily visible to the eye. This framework is of two types, each with many variations, which depend upon the nature and concentration both of the fixative and of the colloidal solution, and to some extent also on temperature and other attendant conditions. These two types, the spongelike and the vesicular, closely correspond respectively to the reticular and the alveolar structures in protoplasm. In the first case the more solid portions form a sponge-like network the interstices of which are occupied by a continuous liquid; in the second case the liquid phase is discontinuous, taking the form of separate drops completely surrounded by the continuous more solid portions.

The sponge-like type appears, for example, in an aqueous 13% solution of egg-albumin when coagulated by various fixatives, the more solid framework forming a fine, regular sponge-like net, with spheroidal granules at the nodes, the diameter of the meshes being least upon fixation by osmic vapor (0.5–0.7μ) and greatest with corrosive sublimate (1.7μ), as shown in Fig. 25. A corresponding difference is seen in protoplasm when fixed by the same two methods. With higher concentrations of albumin the nodal granules are much enlarged and closely crowded; with lower concentrations the net becomes irregular, discontinuous and finally appears as a flaky precipitate, or in the form of fine separate granules.

With gelatin-solutions the results are still more varied. In solutions of 7% to 15% coagulated by formalin an open net is produced, and the same effect appears in solutions of less than 5% coagulated by alcohol or sublimate. If, however, the same stronger solutions be fixed by alcohol or sublimate vesicular or alveolar structure appears, consisting of separate droplets, each completely surrounded by more solid and continuous walls. The diameter of these vesicles is inversely proportional to the concentration

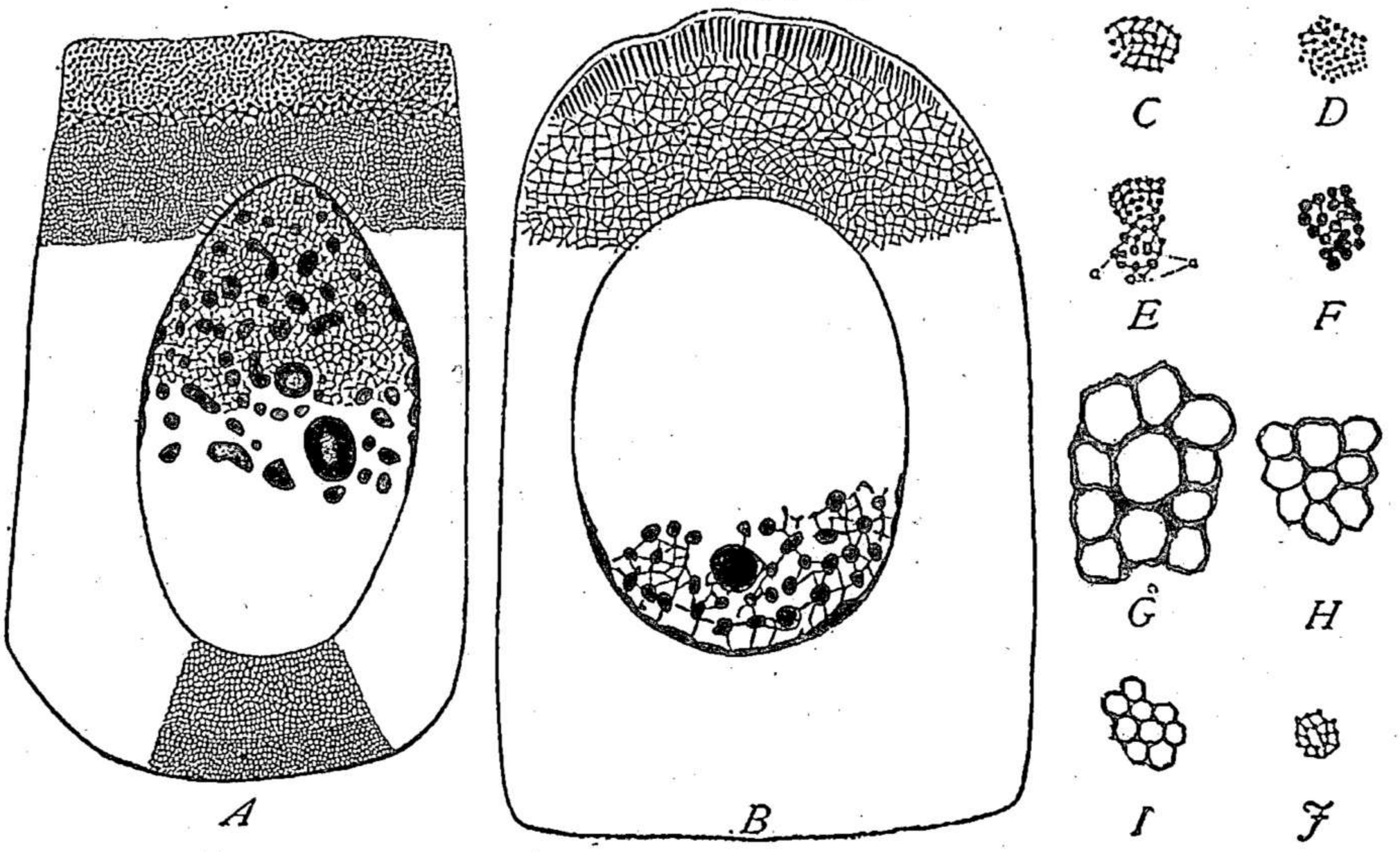

Fig. 25.—Coagulated cells and coagulation-artifacts (HARDY).

A, *B*, epithelial cells, gut of *Oniscus*, *A*, fixed with osmic vapor, *B*, with mercuric bichloride; *C–F*, coagulated egg-albumin; *C*, 13% solids, sublimate; *D*, the same, potassium sulphocyanate; *E*, 30% solids, with included carmine-grains (*a*, *a*), sublimate; *F*, 60% solids, sublimate; *G–J*, coagulated gelatin fixed with sublimate; *G*, 10% solids; *H*, 25% solids; *I*, 50% solids; *J*, 4% solids.

of the solution; after sublimate fixation they range from 7μ (10% gelatin solution) down to 2.5μ (50% gelatin), as shown in Fig. 25. With 4% gelatin solution, however, a network appears with meshes $\pm$ 2μ in diameter. These experiments show how readily reticulated and alveolar formations may be transformed into each other as a result of fixation, the more liquid matter appearing in one case as a continuous substance filling the interstices of a solid sponge-like network, in the other in the form of separate and discontinuous drops with the solid matter forming a continuous honeycomb structure between them.

We are thus made to realize that the results of fixation do not in themselves necessarily give us any information concerning the structure of the original system. We cannot wonder, therefore, that the works of Bütschli, Hardy, Fischer and their predecessors set in motion a pronounced wave of scepticism on the part of cytologists and physiologists concerning the existence of reticular and filar formations in protoplasm, and even led

to a practical denial by some writers that the meshworks or frameworks seen in fixed material have any significance beyond that of coagulation-products. Though this conclusion went too far it served a most useful purpose by putting cytologists on their guard against sources of error in their technique and in reviving interest in the study of living protoplasm.

5. The Alveolar or Foam-Theory of Protoplasm

From the first Bütschli placed a wholly different interpretation upon the protoplasmic meshwork, whether observed in living protoplasm or in the artificial coagulum. Already in his early work (1878) he expressed the opinion that in the Protozoa a gradual transition exists "from protoplasm in which appear simple scattered vacuoles to completely alveolar or, *what is the same thing*, *reticular protoplasm*, where the alveoli are so densely crowded that their protoplasmic walls take on a honeycomb arrangement, which in optical section appears reticular." This was the germ of Bütschli's later conclusion (1892) that protoplasm has everywhere a foam-like or "alveolar" structure, consisting of two principal substances, one continuous and commonly of higher viscosity, and a second that is discontinuous, appearing in the form of separate but often closely crowded *alveolar spheres* suspended in the continuous substance. Both substances were considered by Bütschli as viscid liquids of different physical properties forming an emulsion-like mixture. Later researches have shown, that the viscosity of this mixture varies greatly in different phases of the cell-activities (p. 60), and also that the alveolar spheres or "macrosomes" may in some cases approach the solid state, so as appropriately to be described as "granules." [1] These spheres were commonly spoken of by Bütschli as *alveoli*, though strictly speaking this term applies to the cavities which they fill.

Among the alveolar spheres, likewise suspended in the continuous substance, are numerous minute granules or *microsomes*, fairly uniform in size but to some extent intermingled with smaller ones which graduate down to the limit of microscopical vision (Figs. 26, 27). The microsomes are sharply distinct from the larger spheres or macrosomes in size, staining reactions and sometimes in color [2] even in the living protoplasm. In the eggs of echinoderms, tunicates and other animals the microsomes are rather strongly basophilic and have been regarded by some observers as "chromidia." Van Herwerden ('13) has shown that, like basichromatin, they are readily dissolved by nuclease; but there is little evidence of their derivation from the nucleus.

In many objects, the alveolar spheres are closely pressed together so as to become angular in form, while the interalveolar substance is reduced

[1] *Cf.* Wilson, '99.

[2] *E. g.*, in *Ophiura*, Wilson, '99.

to thin walls or lamellæ between them, as often in an artificial emulsion. Thus arises a foam-like or honeycomb structure in which the microsomes

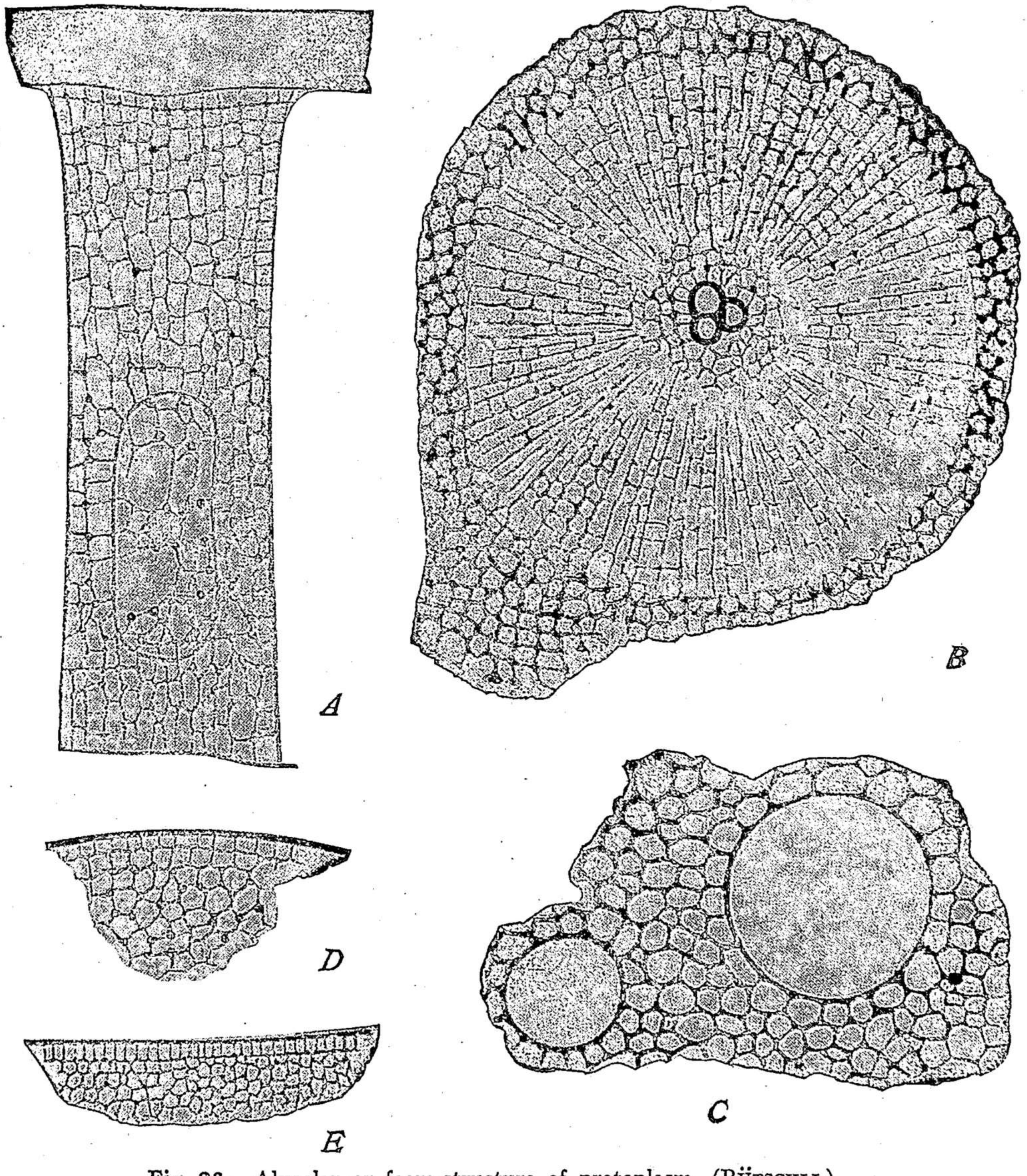

Fig. 26.—Alveolar or foam-structure of protoplasm. (BÜTSCHLI.).

A, epidermal cell of the earthworm; *B*, aster and central bodies from sea-urchin egg; *C*, intracapsular protoplasm of a radiolarian (*Thalassicolla*) with vacuoles; *D*, peripheral cytoplasm of sea-urchin egg; *E*, artificial emulsion of olive-oil, sodium chloride, and water.

tend to collect at the angles where two or more of the lamellæ meet, and which in optical section gives the appearance of a net-like framework or reticulum (Fig. 26).

This general account has been confirmed by many later observers [1] who

[1] *E. g.*, by Erlanger ('96), G. F. Andrews ('97), Rhumbler ('98), Wilson ('99), etc.

have simplified the terminology by applying the old word *hyaloplasm* to the continuous or "interalveolar" substance and *enchylema* to the discontinuous substance of the alveolar spheres (Rhumbler, '98, Wilson, '00). The terminology is thus brought into harmony with that employed for the fibrillar conceptions of protoplasm, and for this reason will hereafter be employed in this work.[1] The microsomes show a tendency to collect at the angles or nodes where two or more of the hyaloplasmic lamellæ meet.

All this was closely imitated by Bütschli ('92) in artificial oil-emulsions in which the part of the hyaloplasm is played by thickened olive oil, that of the enchylema by drops of soapy solutions of mineral salts (*e. g.*, NaCl), and that of the microsomes by particles of soot or carmine suspended in the oil. The artificial alveolar structure thus prepared shows a startling resemblance to that of living protoplasm, which is heightened by the fact that drops of the mixture suspended in water undergo changes of form that may even simulate amœboid movements.

A critical comparison of alveolar protoplasm as seen in the living object and in fixed and stained sections is highly instructive. In the former case (sea-urchin egg) the outlines of the alveolar spheres are readily seen in the living object. In sections, on the other hand, even after the best fixation and staining, the outlines of the spheres are often no longer visible as such (owing to the clearing process), and the eye perceives only a meshwork of microsomes containing crowded clear cavities or alveoli (Figs. 27, 28). With less perfect fixation the alveolar spheres break up or run together in various degrees while the hyaloplasm coagulates in the form of a more or less continuous network (Wilson, '99). It seems certain that many of the so-called reticular formations in protoplasm, as described by earlier observers, arise in this way. A study of such preparations makes it clear that *the "reticulum" is composed of the continuous substance or hyaloplasm*, while the so-called ground-substance, cell-sap or inter-filar substance corresponds to the alveolar substance.

Bütschli considered that the longitudinal "striation" or "fibrillation" seen in muscle-fibers or nerve-fibers, is merely an alveolar structure drawn out into elongated and parallel meshes, while the asters and spindles seen in mitosis were in like manner interpreted as temporary radial configurations of a similar alveolar structure about the division-centers (Fig. 26).

[1] Leydig (1883) used the word "hyaloplasm" in the contrary sense, applying it to the "ground-substance" (nuclear sap, enchylema, or inter-filar substance) as distinguished from the "spongioplasm" or substance of the network or fibrillar formation. Though this usage has been employed even by some recent writers (*e. g.*, Conklin '17) it seems to the author to be out of harmony with the general historical development of the subject as affected by Bütschli's theory, and to introduce needless confusion.

Both appearances were successfully imitated in the artificial emulsion; and Bütschli also produced very striking amphiastral figures by coagulating hot gelatin containing scattered air bubbles. About the latter conspicuous asters are formed while adjoining bubbles become connected by conspicuous

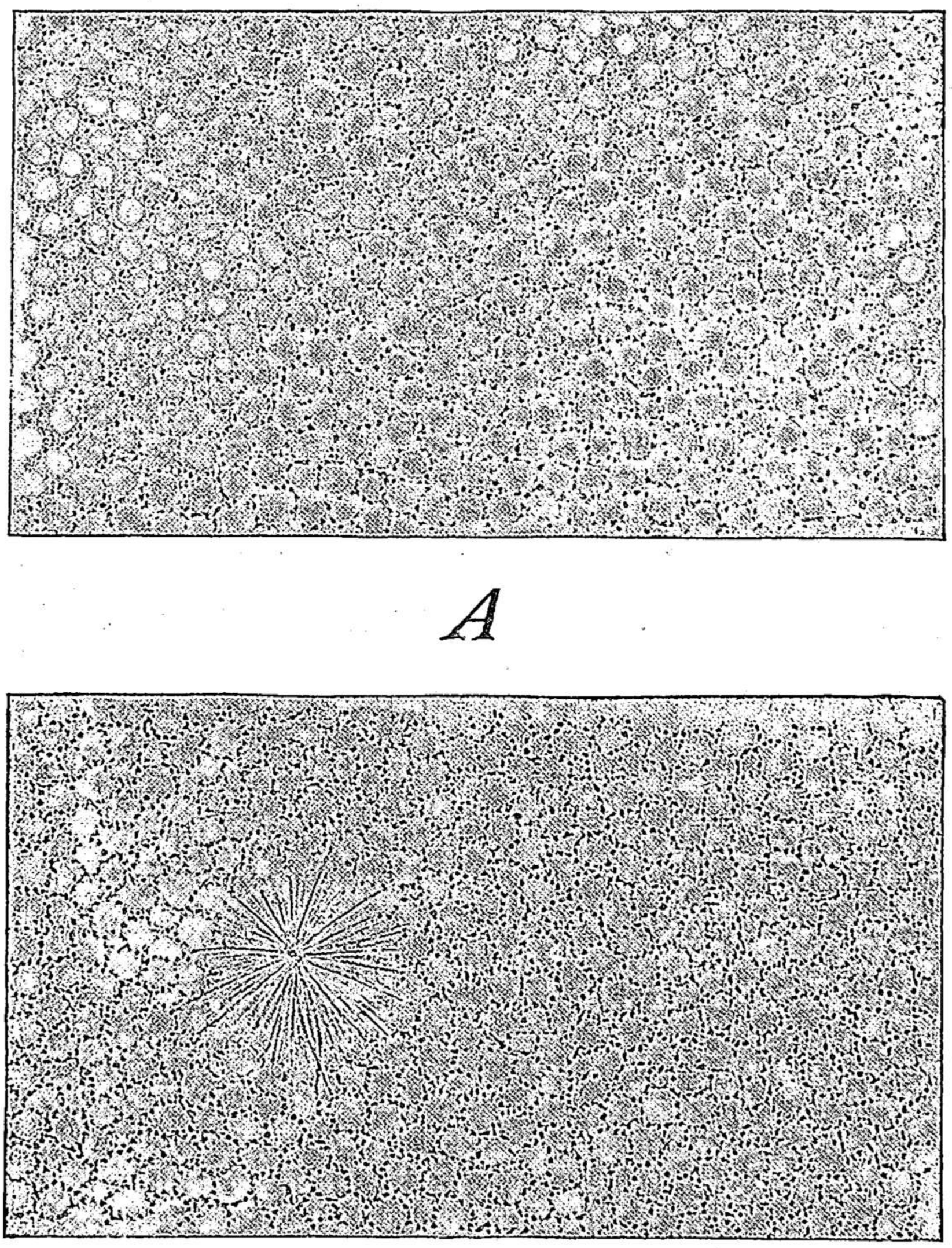

Fig. 27.—Structure of protoplasm.

A, alveolar structure, living protoplasm of the starfish *Asterias* showing alveolar spheres (megasomes) and microsomes; *B*, the same after fixation (sublimate-acetic), a small sperm-aster and central body towards the left.

spindles. Bütschli also brought forward evidence that protoplasm which appears to be quite hyaline or homogeneous may nevertheless possess an alveolar structure that is invisible because of the extreme tenuity of the inter-alveolar walls.

Bütschli was careful to distinguish between the "primary" or "funda-

mental" alveolar structure and the "secondary" or derived structures that may arise through the appearance of larger vacuoles or other inclusions in the protoplasm. In the former, which he considers to be a universal characteristic of protoplasm, the alveoli are not more than 1.5–2.0 microns in diameter. All coarser structures arising through the deposit of larger drops, or granules ("pseudalveolar structures" of Reinke) are of secondary origin and inconstant occurrence. Bütschli offers evidence in later experimental studies ('98, etc.), that the meshworks seen in coagulated colloids, especially in gelatin, are often not true networks but alveolar formations which he compares directly with the gel phase of the colloids (*cf.* p. 66).

6. Critique of Bütschli's Theory

There can now be no doubt that protoplasm exhibits in many cases the structure described by Bütschli; but even his strongest supporters are now convinced that he, like many another reformer, pushed his conclusions too far. In the first place, the evidence that true fibrillar formations exist in protoplasm has become irresistible.[1] This conclusion rests in part upon the extreme clearness with which such formations can be demonstrated, for instance, in nerve-cells (Bethe, Apathy, Cajal, Dogiel, etc.), or columnar epithelial cells (Heidenhain, Del Rio, etc.); in part on histogenetic studies, particularly on muscle-cells, in which the formation and growth of the fibrillar formations, step by step, has been minutely studied (*e. g.*, Heidenhain, '99, Godlewski, '01, Duesberg, '09). Again, recent studies on chondriosomes have most clearly demonstrated the existence in nearly all kinds of cells of those specific forms of fibrillæ known as *chondrioconts*, and have given ground for the conclusion that from them some of the more specialized types of fibrils (myofibrillæ, etc.) may be derived (p. 707). The chondrioconts were long since seen in the *living* cells of cartilage and other tissues by Flemming ('82), and undoubtedly form an important part of the filar formation or "mitome," as described by him.[2]

The existence of fibrillæ in the protoplasmic substances is by no means incompatible with the alveolar theory and many observers have urged that both types of structure may coexist side by side. Strasburger, for example ('92), whose views have been followed by many botanists, considers the cytoplasm to consist in general of two physiologically different plasms which differ characteristically both in structure and in function, one being an especially nutritive or vegetative *trophoplasm*, typically alveolar in structure, the other a more active *kinoplasm* especially concerned with

[1] See especially Heidenhain's great work on Plasma und Zelle ('07, '11), in which these formations are exhaustively treated.

[2] See Meves '10 b.

movement (cilia, etc.), cell-division and irritability, and typically fibrillar in structure ("filar plasm" as distinguished from "alveolar plasm").[1]

In the second place, it is more than doubtful whether Bütschli's "finer" or "true" alveolar structure is a primary or universal characteristic of protoplasm; and whether it is logically separable from the coarser or "secondary" structure ("pseudo-alveolar structure" of Reinke). In the sea-urchin eggs, a classical example of the alveolar structure, it may very

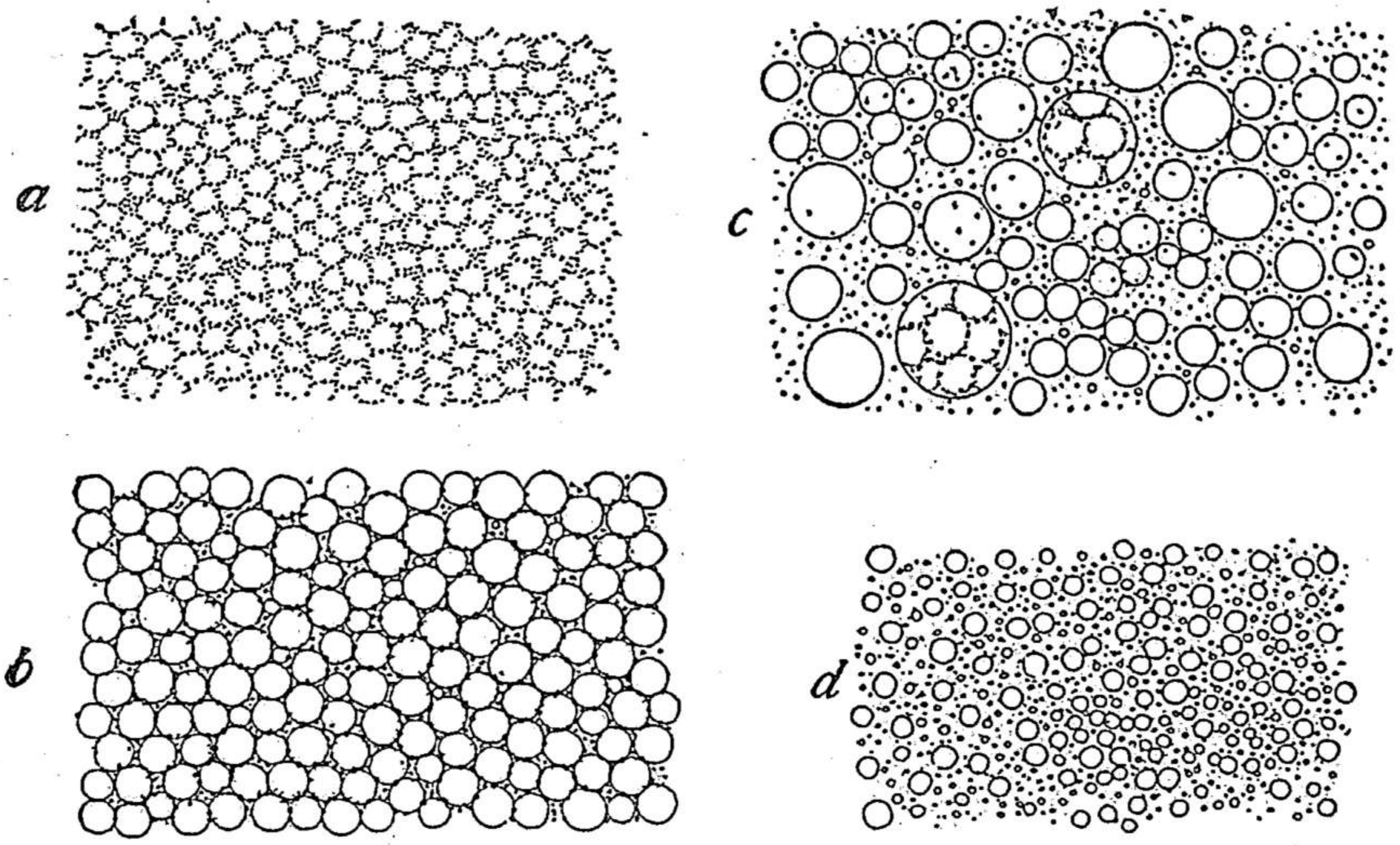

Fig. 28.—*a*, protoplasm of the egg of the sea-urchin (*Toxopneustes*) in section showing meshwork of microsomes; *b*, protoplasm from a living starfish egg (*Asterias*) showing alveolar spheres with microsomes scattered between them; *c*, the same in a dying condition after crushing the egg: alveolar spheres fusing to form larger spheres; *d*, protoplasm from a young ovarian egg of the same (all the figures magnified 1200 diameters).

clearly be seen, both in the living material, and in sections, that this structure is of secondary origin (Wilson, '99.) The very young ova consist largely of hyaline protoplasm or hyaloplasm, with only a few scattered "granules." Step by step as the egg grows the granules increase in number and the alveolar spheres emerge into view, at first very minute and scattered, later growing more numerous and larger until they crowd together to form the alveolar structure as described by Bütschli; though in the forms studied by the author the alveolar spheres do not flatten together to the extent figured by him. The observations of the writer also showed that the "microsomes," like the alveolar spheres, may be liquid drops; and also, in conformity with the earlier conclusion of G. F. Andrews (1897), that the "continuous" or interalveolar walls may themselves show a still finer alveolization down to the limits of microscopical vision.

Comparative studies show that it is practically impossible to draw any clear or logical line of distinction between the "true" or "fundamental"

[1] *Cf.* p. 633.

alveolar structure and the coarser "secondary" (pseudo-alveolar). Such facts point to the conclusion, that "we are probably justified in regarding the continuous substance (*i. e.*, the hyaloplasm) as the most constant and active element, and that which forms the fundamental basis of the system, transforming itself into granules, drops, fibrillæ or networks" [1] in different phases of its activity. This opinion is in principle shared by Heidenhain ('07), Conklin ('12) and others.[2]

Bütschli's conception of protoplasmic structure is essentially that of a complex colloidal system. The genesis of the alveolar structure in the ovarian egg, as above described, leads us to conclude that it is similar in type to the invisible structure of a colloidal solution or suspension. The combined cytological and physico-chemical evidence thus seems to justify the conclusion that in protoplasm, as in other colloidal systems, the discontinuous phase (or phases) may show all degrees of dispersion from very large molecular aggregates (as in the coarser "pseudalveolar" formations, through successively smaller ones down to ultra-microscopical "particles," molecules and ions.[3] We may thus conceive Bütschli's structure as arising in the hyaloplasm either by growth or by successive aggregations of particles which ultimately become visible in the form of suspended granules, microsomes and alveolar spheres or macrosomes. If this be correct the visible alveolar structure differs from that of the apparently homogeneous hyaloplasm only in degree, and a consistent view of the whole series of phenomena is attained. For the cytologist, however, it is essential to keep always in view the fact that artificial preparations are coagulation-products which may depart more or less widely from the conditions existing in life.

7. The Granule Theory of Protoplasm

We may here briefly consider a speculative conception of protoplasm which, though long discredited, still offers many interesting suggestions for the general problems of cell-organization. It was suggested by several earlier observers [4] that the protoplasmic granules might be regarded as organic units ("plastidules," etc.) which build up the cell somewhat as cells build up the multicellular body; and this speculation was at least brought within the range of possibility by the remarkable studies of Schimper ('85) on the plastids of plant-cells, which showed that these bodies independently grow and divide, like symbiotic organisms within the cell,

[1] Wilson, '00, p. 50.

[2] *Cf.* Heidenhain, 1907, p. 489.

[3] *Cf.* Bayliss. "There is no hard and fast line to be drawn between matter in pieces visible to the naked eye, down through ultra-microscopical particles to molecules." *The Nature of Enzyme Action*, p. 201, 1911.

[4] Henle ('41), Béchamp and Estor ('60), Maggi ('78).

and that in higher plants the plastids of the adult tissue-cells arise in this manner from minute plastids present in large numbers in the embryonic cells and even in the egg. The granule-theory first appears in clearly defined form, and based on more extended observation, in the works of Altmann ('86, '90, '94) and underwent further systematic development by J. Arnold, Schlater, Rohde, St. Hilaire, C. Schneider, and many others including Benda, Meves, and other leaders in the modern theory of the chondriosomes (pp. 47, 717).

Altmann, making use of a special technique,[1] was able to demonstrate fuchsinophilous (red-staining) granules in many kinds of cells, often nearly

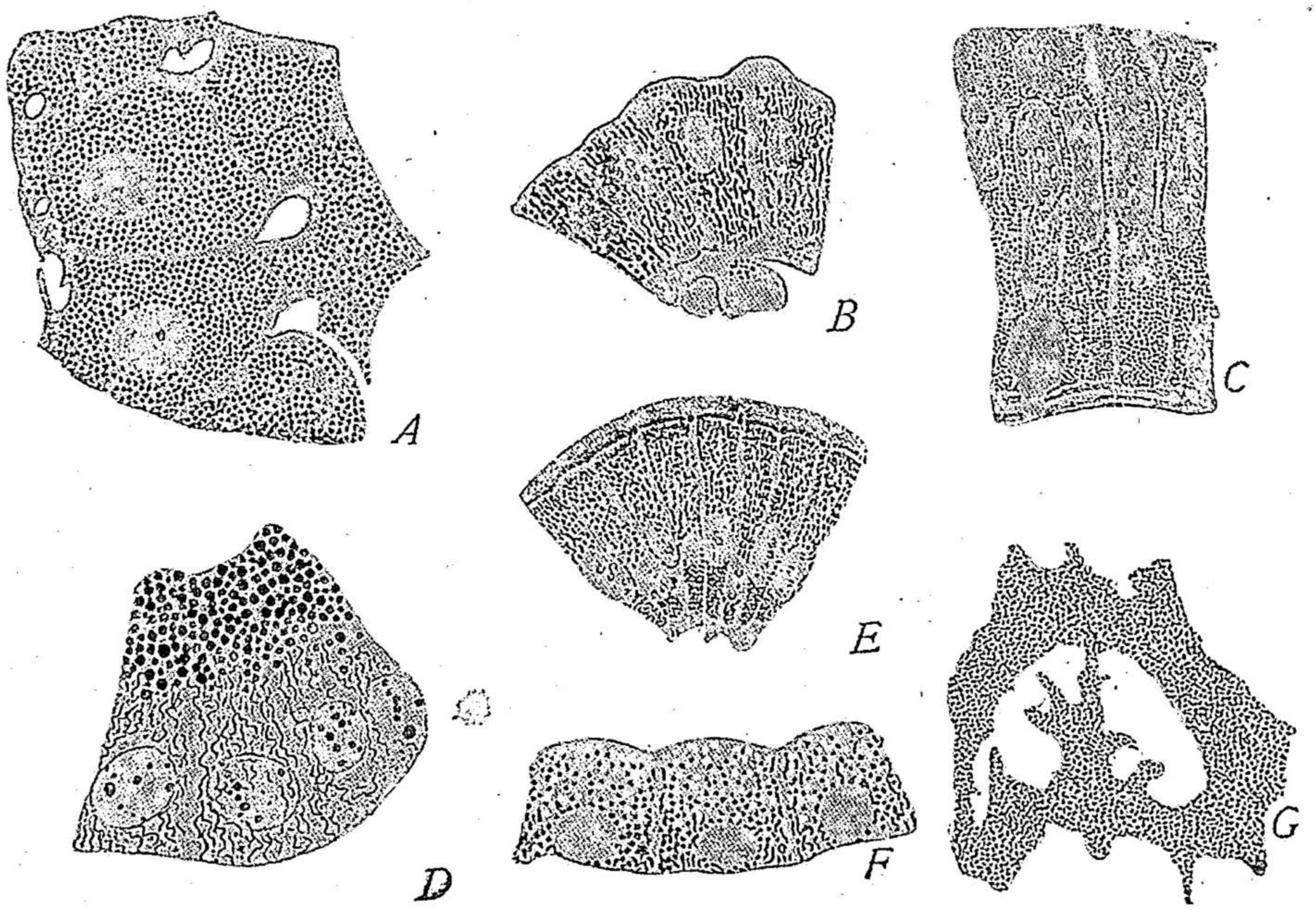

Fig. 29.—Granular structures as figured by Altmann after various modes of fixation and staining by acid fuchsin and picric acid. Many of these are now known to be mitochondria (ALTMANN).

A, liver of the mouse; *B*, tubules of mesonephros, embryo chick; *C*, intestinal epithelium of frog; *D*, pancreas of *Triton*, showing secretory granules and fibrillæ; *E*, epithelium of intestinal villus, cat; *F*, Harderian gland, rabbit; *G*, small portion of pigment-cell with pigment-granules, salamander larva.

uniform in size, and in many cases so closely crowded as almost to constitute an alveolar structure like that of Bütschli (Fig. 29). These granules were regarded by Altmann as "elementary organisms" ("bioblasts," "cytoblasts") or their products, which live in a homogeneous basis or ground-substance. He pointed out the analogy between such a structure and that

[1] Fixation by potassium bichromate and osmic acid; staining with acid fuchsin and picric acid, by which the granules are stained intensely red. This method has been developed by Meves, Bensley and other more modern students of the mitochondria.

of a bacterial zoöglœa, further suggesting that the granules might in some cases even live separately in the form of minute microörganisms of the *Micrococcus* type. The granules were assumed to arise only by the growth and division of preëxisting granules—*omne granulum e granulo* (1890)—after the fashion of plastids; and Altmann regarded them as the essential living units of protoplasm out of which every living part of the cell is built.[1] Admitting that many of these granules (secretory granules, etc.) belong to the passive or metaplasmic elements, Altmann regarded such granules as products of originally living granules or bioblasts. This is quite analogous to the formation of non-living products by entire cells and, as will be seen, is nearly akin to more recent views concerning the chondriosomes. Altmann did not hesitate to push his conception beyond the visible structure of protoplasm into that which lies beyond the reach of the microscope; but this side of the question may better be considered at a later point (p. 717). His conclusions were insecurely based, and at first gained few adherents, in part because of their too speculative character, in part because of his failure to distinguish sufficiently between structures that preëxist in the living cell and those that are products of the coagulating effect of fixing agents. Continued studies on the protoplasmic granules, especially as seen in *vivo*, nevertheless led many competent observers to a somewhat more favorable judgment concerning the essential features of his theory, though it still remains a subject of controversy.[2] It is now generally admitted that many forms of granules play an active and important part in the protoplasmic activities and are not to be regarded as merely coagulation-artifacts, or metaplasmic products. The main controversial questions relate to their morphological nature and origin. A prominent place in the study of this question has been taken by J. Arnold ('79, '07, '14, etc.) who believes that the so-called "inter-granular substance" of earlier writers (hyaloplasm) is largely made up of very minute but still visible granules or "*plasmosomes*" and fibrillæ ("*plasmomites*") by the enlargement of which may be derived many of the larger formed elements; and a somewhat similar view is advocated by Heidenhain ('11) who follows Altmann in the conclusion that the smallest visible granules (plasmosomes) may themselves arise by enlargement of still smaller invisible metastructural bodies. To this question we shall later return (p. 717). Here we only draw attention to the prominence recently given to the granule-theory by the researches of Benda, Meves and their followers, who have urged the identity

[1] "Protoplasm may be defined as a colony of bioblasts, the individual elements of which are grouped like those of a zoöglœa or in filamentary chains, and held together by an indifferent substance." Altmann, '94, p. 140.

[2] Literature especially in Heidenhain ('11) and J. Arnold ('14). See also earlier works of Arnold ('98, '00, '07b, '13a, etc.), Schlater ('95, '03, '11), Rohde ('14, etc.).

of the mitochondria (p. 47) with Altmann's granules and ascribed to them a position of fundamental importance in the cell-activities.

Leaving all theory aside, Altmann's objective description of the structure of protoplasm to a certain extent approaches that of Bütschli as modified by G. F. Andrews and the author, save that the "granules" were assumed to be of more solid consistency than the alveolar spheres or the microsomes (pp. 72, 73). The recent experimental studies of Kite and others on living protoplasm gives considerable reason to regard the alveolar spheres as of rather firm consistency, even in the echinoderm egg (one of Bütschli's principal objects); while a number of observers have actually described them as "granules." The physical consistency of the granules or drops seems, however, a matter of secondary importance in view of the readiness with which the protoplasmic colloids may undergo changes of physical consistency (*cf.* p. 60). In another direction Altmann's theory comes into relations with the filar theory of Flemming; for Altmann held that the granules might grow out into rods or fibrillæ or produce such structures by a process of linear alignment; and this is borne out by recent studies on the chondriosomes, as will later be explained. Whatever be its points of weakness on the physiological and theoretical side, therefore, the granule-theory opens the way to a reconciliation between opposing views on protoplasmic structure so widely divergent as at first sight to offer a total and fundamental contradiction; [1] while the contradiction between it and the colloidal nature of the cell-substance is I believe wholly illusory.

Summary on Protoplasmic Structure

Up to the present time no single theory of protoplasmic structure has commanded general acceptance, and it is more than doubtful whether any universal formula for this structure can be given. We are driven by a hundred reasons to conclude that protoplasm has an organization that is perfectly definite, but it is one that finds visible expression in a protean variety of structures, and we are not in a position to regard any of these as universally diagnostic of the living substance. As far as *visible* structure is concerned no satisfactory distinction, practical or logical, in the opinion of the author, can be drawn between a "primary" or "fundamental" structure, and a secondary one. The fundamental structure of protoplasm lies beyond the present limits of microscopical vision and hence still remains a matter of inference and hypothesis. Probably the only element of protoplasm that will be admitted by all cytologists to be omnipresent is the "homogeneous" hyaloplasm, which offers to the eye no visible structure. Almost always, however, protoplasm exhibits a visible structure owing to

[1] See especially Meves ('10b).

the presence in the hyaloplasm of alveolar, reticular, fibrillar or granular formations; but these vary widely in different kinds of cells, at different periods of development, and in different phases of physiological activity.

We are not in a position to characterize any of these elements as "living" in contradistinction to "lifeless" constituents of the protoplasm. Nevertheless, there is reason to conclude that of all the cell-constituents the "structureless" hyaloplasm is the most constant and most active; and may perhaps be regarded as forming the fundamental basis of the protoplasmic system from which directly or indirectly, all other elements take their origin. Such a view, it is true, does not yet command the acceptance of many cytologists, yet it involves a minimal amount of theory and is fully in harmony with those physicochemical studies that have proved the cell-substance to have in many cases the properties of a colloidal system. This conclusion, it is true, throws us back upon the assumption of a "meta-structure" in protoplasm that lies beyond the present limits of microscopical vision; but in this respect the biologist is perhaps in no worse case than the chemist or the physicist.[1]

IV. THE NUCLEUS

As seen in the living cell the nucleus most commonly appears as a clear, rounded, sac-like body bounded by a delicate membrane and often showing no visible structure save for the presence within it of one or more smaller rounded bodies, *the nucleoli*. After coagulation by fixing agents, the nucleus offers a much more complicated appearance, containing in addition to the nucleoli a net-like framework (Fig. 6) in which are suspended granules or irregular clumps composed of a substance that stains intensely with certain dyes (in particular the basic coal tar colors such as methyl-green or safranin), and hence from the time of Flemming (1879) has been widely known as *chromatin*.

The form of the nucleus is on the whole singularly constant as compared with that of the cytosome, and shows little correlation with the latter; but it is a familiar fact that long cells, such as muscle-cells, columnar epithelial cells (Figs. 17, 42) or certain forms of parenchyma, usually have more or less elongated nuclei. Typically rounded and with an even contour, it may in certain cases become irregular and has often been observed, in particular cases, to undergo slow amœboid changes of form in the living cell, *e. g.*, in cartilage-cells, leucocytes, or animal ova. Nuclei of irregular or amœboid form are frequent in cells characterized by very active metabolism, in which case the nuclei are often not only of large size but show a marked

[1] Further evidence on this question will be presented later. (See p. 717.)

further increase of surface by the formation of lobes, sacculations, or even, in extreme cases, of complex branches ramifying through the cell. An extreme example of this is offered by the spinning glands of certain insect-larvæ (Lepidoptera, Trichoptera) in which the nucleus, originally spheroidal, finally assumes a labyrinthine appearance with convolutions occupying a large area in the cell (Figs. 31, 34). In other cases the nucleus shows deep infoldings or incisions and sometimes even tubular ingrowths of membrane forming intra-nuclear canaliculi; and it has been shown that such infoldings may unfold or evaginate, thus increasing the nuclear size.[1]

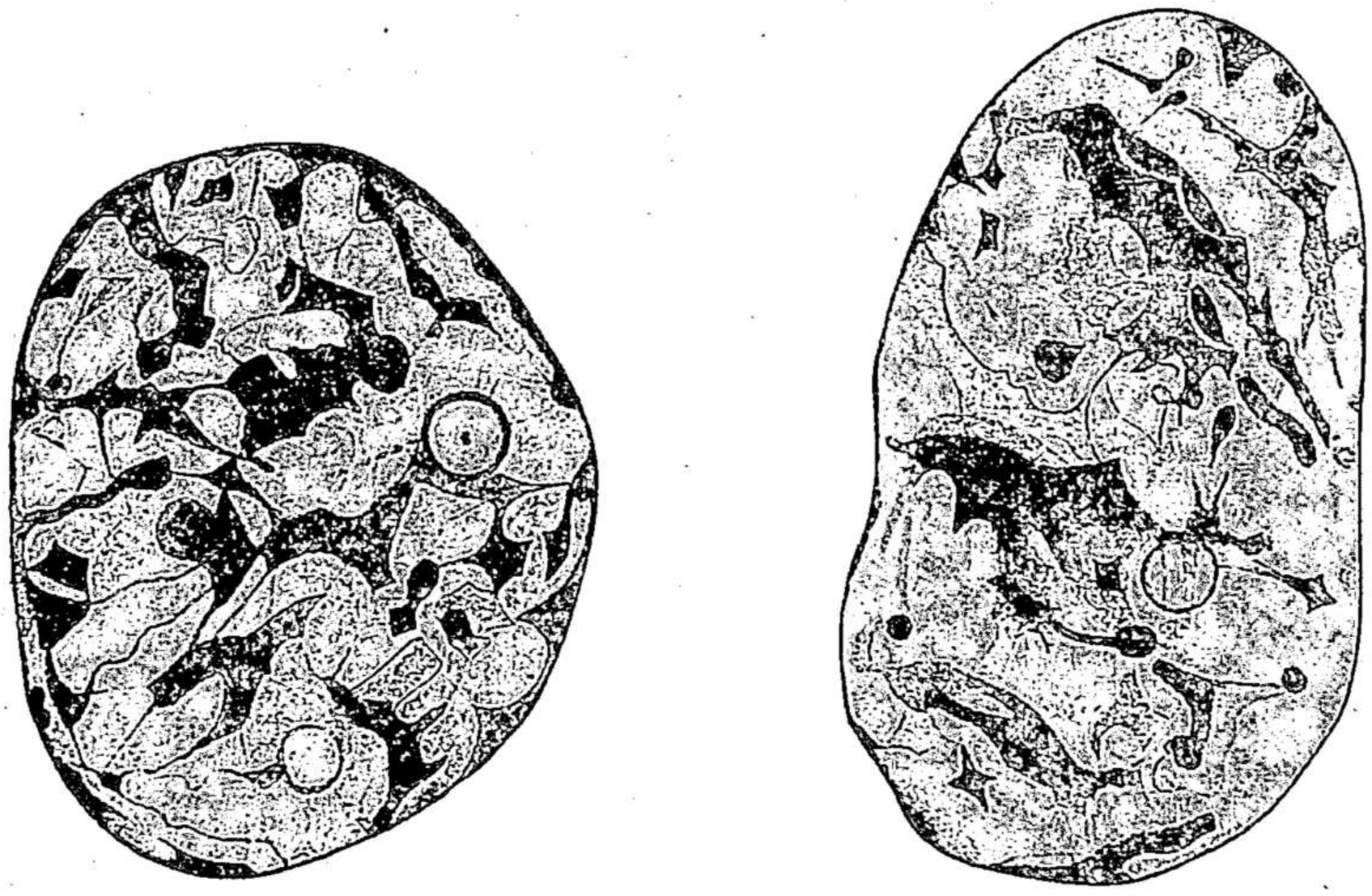

Fig. 30.—Two nuclei from the crypts of Lieberkühn in the salamander (HEIDENHAIN).

The character of the chromatin-network (basichromatin) is accurately shown. The left nucleus contains three plasmosomes or true nucleoli; the right, one. A few fine linin-threads are seen in the left nucleus running off from the chromatin-masses. The clear spaces are occupied by the ground-substance or nuclear sap.

In certain types of cells the surface of the nucleus may also be increased by its breaking up into more or less separate vesicles or karyomerites, thus forming "polymorphic" nuclei or nuclear nests. Nuclei of this type are in some instances morphological multiples resulting from a process of true nuclear division without cytoplasmic division (*e. g.*, in case of the ring-nuclei of giant-cells, Fig. 34, or of certain kinds of leucocytes). More commonly, perhaps, the karyomerites are partial structures due to incomplete union of the chromosomes after cell-division or to amitotic fragmentation of the nucleus (p. 221). Such facts add to the evidence that active exchanges of material between nucleus and cytosome take place in metabolism. In respect to the relative volumes of nucleus and cytosome each type of cell tends towards a certain norm, the *karyoplasmic* ratio (R. Hertwig) (p. 727).

[1] See Champy, '13, Champy and Carleton, '21.

The nuclear substance, considered as a whole, is of colloidal nature like the cytoplasm, and varies widely in physical consistency. In some cases (*e. g.*, in sea-urchin eggs) its substance as a whole shows the properties of a liquid, while the membrane by which it is bounded is very viscous and tough. This is shown by the quick and complete collapse of the nucleus when crushed or cut, while the membrane may remain nearly intact (Kite, '13). That the interior mass of the nucleus often is liquid is proved in several other ways. Nuclei may readily fuse together, either within the cell (as in the fertilization of the egg) or outside the cell when isolated in the fresh condition, as observed by Albrecht ('99). Chambers ('16) has more recently shown that the nucleus of the egg (in sea-urchins) may be cut in two, with the micro-dissection needle, and that the fragments will round up to form spheroidal droplets which will again fuse to form a single normal nucleus. After such an operation the egg is stated still to be capable of normal fertilization and cleavage. Nevertheless the consistency of the nuclear substance as a whole often shows a high degree of viscosity, as shown by the usual absence of Brownian movement and also by microdissection. Kite, for instance, found in some Protozoa (*Amœba*, *Paramœcium*), and in certain metazoan tissue-cells (muscle, epidermis) that the nuclear substance was of firmer consistency like that of a "gel," though not a very solid one.

In fixed preparations the nuclear substance is of course in the main an artificial and semi-solid coagulum. How far the nuclear framework that it contains corresponds to the conditions preëxisting in life is a difficult question. Very often no trace of the framework is seen before coagulation sets in; and this has led to a sceptical attitude concerning it on the part of some observers. On the other hand, living nuclei sometimes show certain well-marked structures in addition to the nucleoli. Flemming ('76–'82) clearly demonstrated this in epithelial cells, cartilage-cells, connective-tissue-cells and leucocytes, showing that the coarser features of the framework are distinctly visible in life, and that they conform closely in form and extent to those that appear in fixed material; and although these observations were later disputed (see especially Tellyesnicky, '05) they have since been confirmed by a number of competent observers. [1] As a rule the only portions of the framework visible in life are the clumps or net-knots of basichromatin, and it is still rather uncertain how far the finer framework may not be a coagulation-effect. Gross ('17) found that some types of living nuclei show in addition to true nucleoli only numerous small granules or microsomes (salivary glands of *Lymnæa*, germinal vesicle of *Anodonta* and *Unio*). In other types, including the epithelial cells of salamander larvæ

[1] See Heidenhain ('07), p. 113.

(Flemming's original object) the living nuclei show in addition to such granules very distinct net-knots. In both cases (as earlier described by F. R. Lillie, '06, in the eggs of *Chætopterus*) the small granules ("microsomes") are said to show active Brownian movements, a fact used by Gross as an argument against the existence of a nuclear network in life. On the other hand, Lundegårdh ('12) figures and describes the living nuclei in root-tips

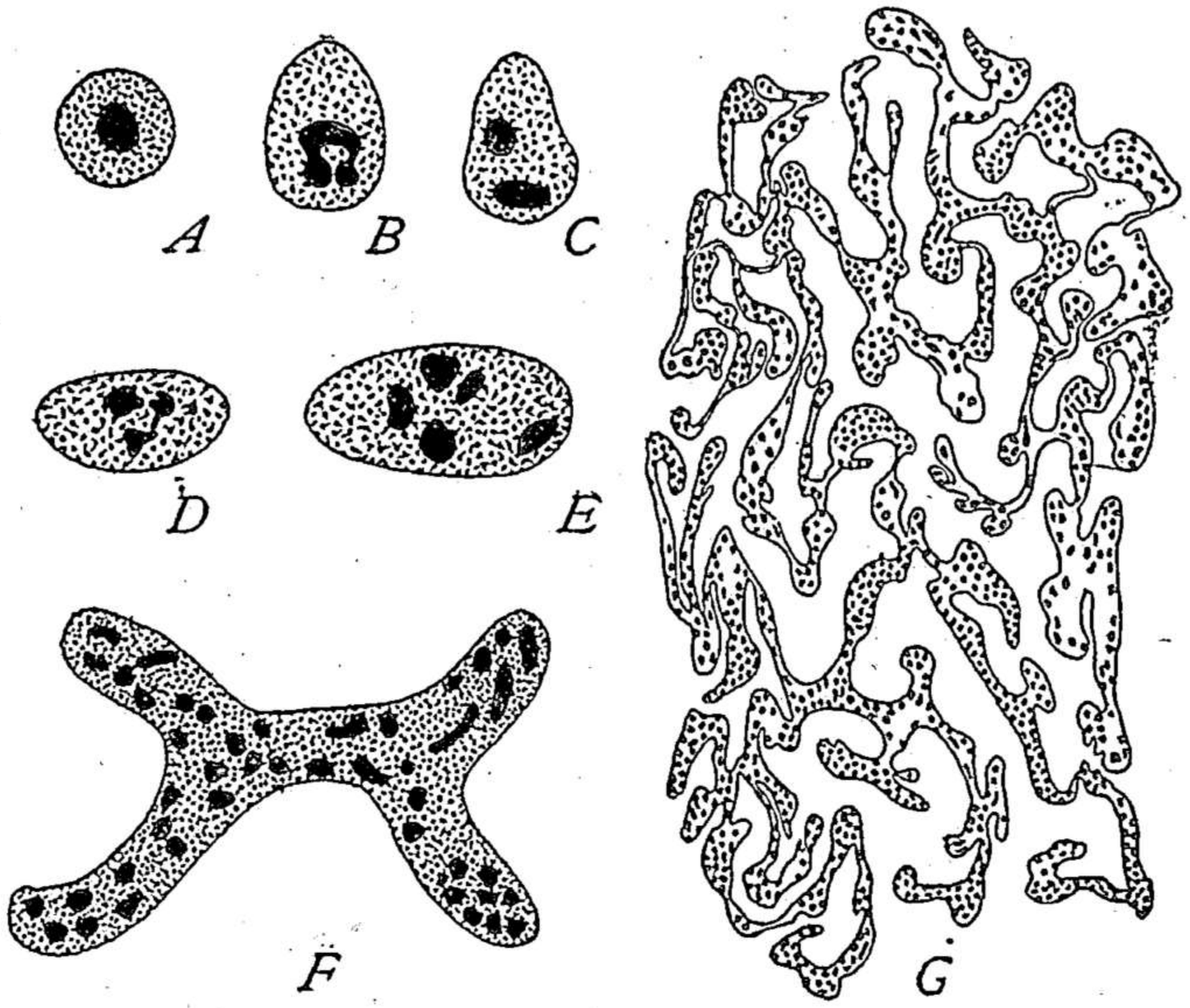

Fig. 31.—Nuclei of spinning glands in the insect *Platyphylax* (VORHIES).

A–F, young nuclei, showing multiplication of nucleoli and beginning of branching; *G*, mature labyrinthine nucleus with numerous nucleoli.

of *Allium* and *Vicia* as filled with small bodies which he considers as "drops" crowded together to form a "granular-alveolar" structure, but also, he insists, anastomosing to form a net-like framework. Such an account seems rather contradictory unless the "drops" be at least semi-solid. [1] The strongest evidence of the preëxistence of some kind of nuclear framework is, however, the gradual formation from it, during mitosis of the spireme-thread, a process long since observed in the living object by both Flemming and Strasburger and since repeatedly traced out with minute care by many observers (Fig. 52). It seems certain that this thread is formed from the more solid portion of the nuclear substance (including the net-knots) and that the apparent absence of structure so often observed in living nuclei is deceptive, being due to a lack of differences of refractive index sufficient to make the formed components of the nucleus visible. It must be confessed, however, that we are not yet in a position to state precisely the rela-

[1] Bütschli long since pointed out the unstable character of a liquid or even viscid network. *Cf.* p. 68.

tion between the preëxisting nuclear framework of the living nucleus and the net-like structure seen in sections.

A. General Structure

In a general way we may distinguish (a) *vesicular*, (b) *massive*, and (c) *chromidial* or *scattered* nuclei; but these are connected by many transitional forms.

The most common type of nucleus is the vesicular, which is of general occurrence in the tissue-cells of most multicellular animals and plants and

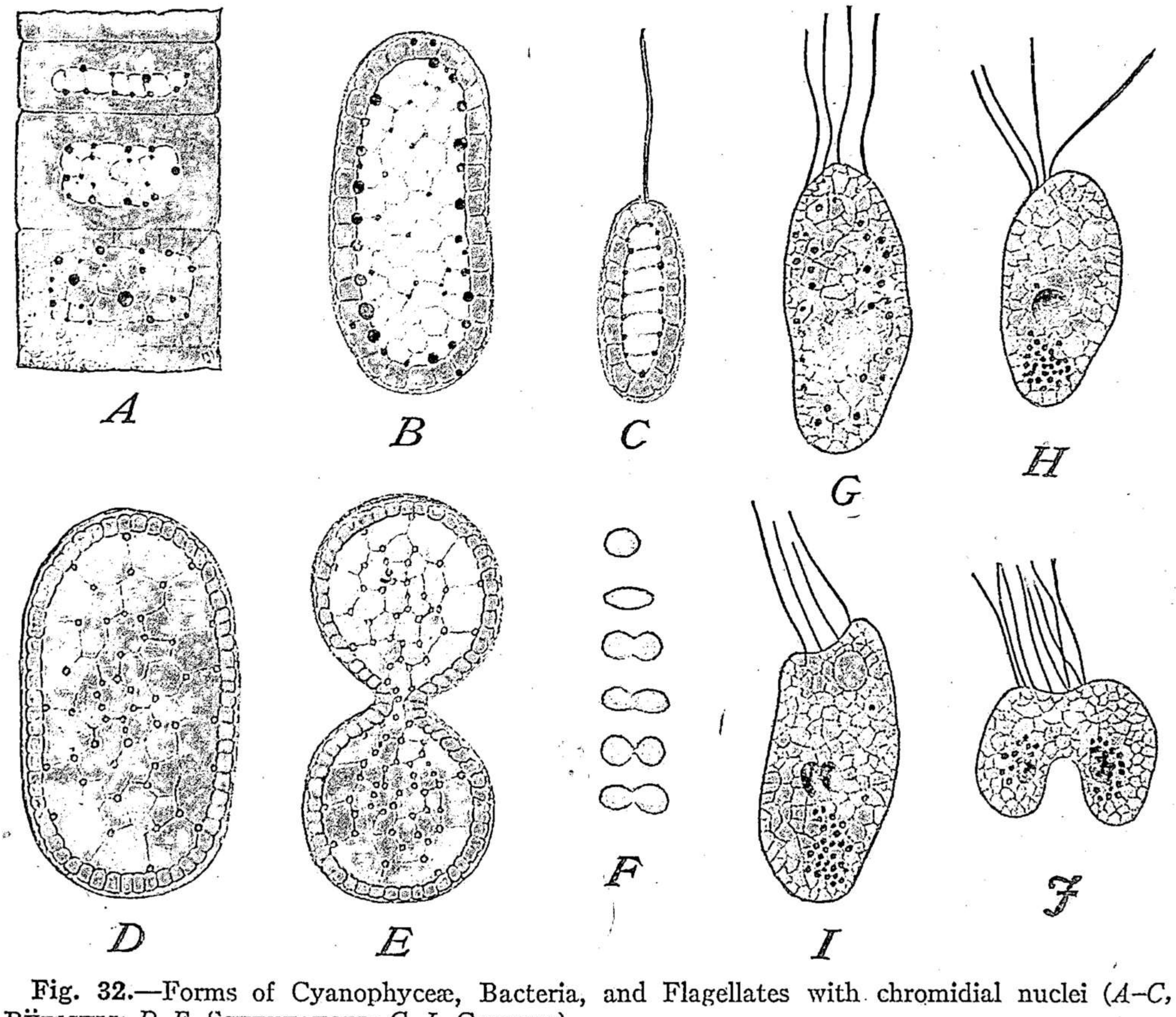

Fig. 32.—Forms of Cyanophyceæ, Bacteria, and Flagellates with chromidial nuclei (*A–C*, Bütschli; *D–F*, Schewiakoff; *G–J*, Calkins).

A, *Oscillaria; B*, *Chromatium; C*, *Bacterium lineola; D*, *Achromatium; E*, the same in division; *F*, supposed stages of fission of the granules; *G*, *Tetramitus*, with central sphere and scattered granules; *H*, aggregation of the granules; *I*, division of the sphere; *J*, fission of the cell.

is also frequent among the Protista. The nucleus of this type is usually if not always bounded by a definite wall or membrane, and contains a sponge-like framework of which the most conspicuous element is the so-called "*chromatin.*" Among Protozoa nuclei of this type often contain a more or less massive central body, the *endosome*, or *karyosome*—some-

times several such—in which all or some of the chromatin may be concentrated; and within it may be contained a still smaller centriole (Figs. 87, 88).[1]

Massive nuclei occur typically in the male germ-cells of animals generally and of many lower plants. Such nuclei usually appear homogeneous and stain with great intensity in basic dyes; but this condition is connected with the more usual one by transitional forms. Nuclei of this type, or approximating to it, are common also among Protista, for example, in the ciliates generally; but in most of the latter forms suitable staining reveals the presence of a very fine chromatic framework. The chromidial nuclei (Figs. 14, 32, 33) are represented by small granules (chromidia or chromioles) or larger irregular clumps of chromatin or a related substance, scattered through the protoplasm without forming a single individualized body.[2] Such a condition can be called a "nucleus" only as a matter of convenience, since this term properly applies only to cases in which the nuclear substance is aggregated to form an individualized body. A permanent chromidial condition of the nucleus is unknown among true multicellular organisms, and exists only in certain special cases among the Protista of which the best determined seem to occur in certain rhizopods, ciliates, bacteria and blue-green algæ. Considerable doubt still exists in regard to these cases, owing to the present lack of any decisive microchemical tests for "chromatin."[3] These doubts are, however, in large measure removed by morphological evidence which shows that in some species the scattered chromidia become aggregated to form a nucleus-like body in preparation for spore-formation (bacteria), division or conjugation. Similar evidence is afforded by those cases in which the scattered or chromidial nucleus is a temporary formation, derived by the breaking down of an ordinary nucleus (or by elimination of chromatin from it) and destined to reform such a nucleus (or nuclei), as has been described in some rhizopods (*Arcella*, Fig. 342, *Arachnula*, Fig. 343). An interesting problem is offered by the blue-green algæ (Cyanophyceæ) a group in which the presence or absence of a nucleus has long been a subject of debate.[4] Most students of this group have found evidence of a more or less diffuse condition of the nuclear substance in the form of scattered, deeply staining granules of "chromatin," "metachromatin" (volutin) or a related substance which, however, show a tendency to collect in the central region of the cytosome.

[1] By some writers this type of nucleus is characterized as a "karyosome-nucleus" in contradistinction to the vesicular type.

[2] *Cf.* p. 700.

[3] *Cf.* pp. 644, 650.

[4] For literature see especially Bütschli ('02), Olive ('05), Guilliermond ('05, '06), Fischer ('05), Gardner ('06), Zacharias ('07), Acton ('14), Baumgärtel ('20).

Thus arises a more or less definite "central body" (Bütschli) or "centroplasm" (Fischer) which by most recent observers is considered as a primi-

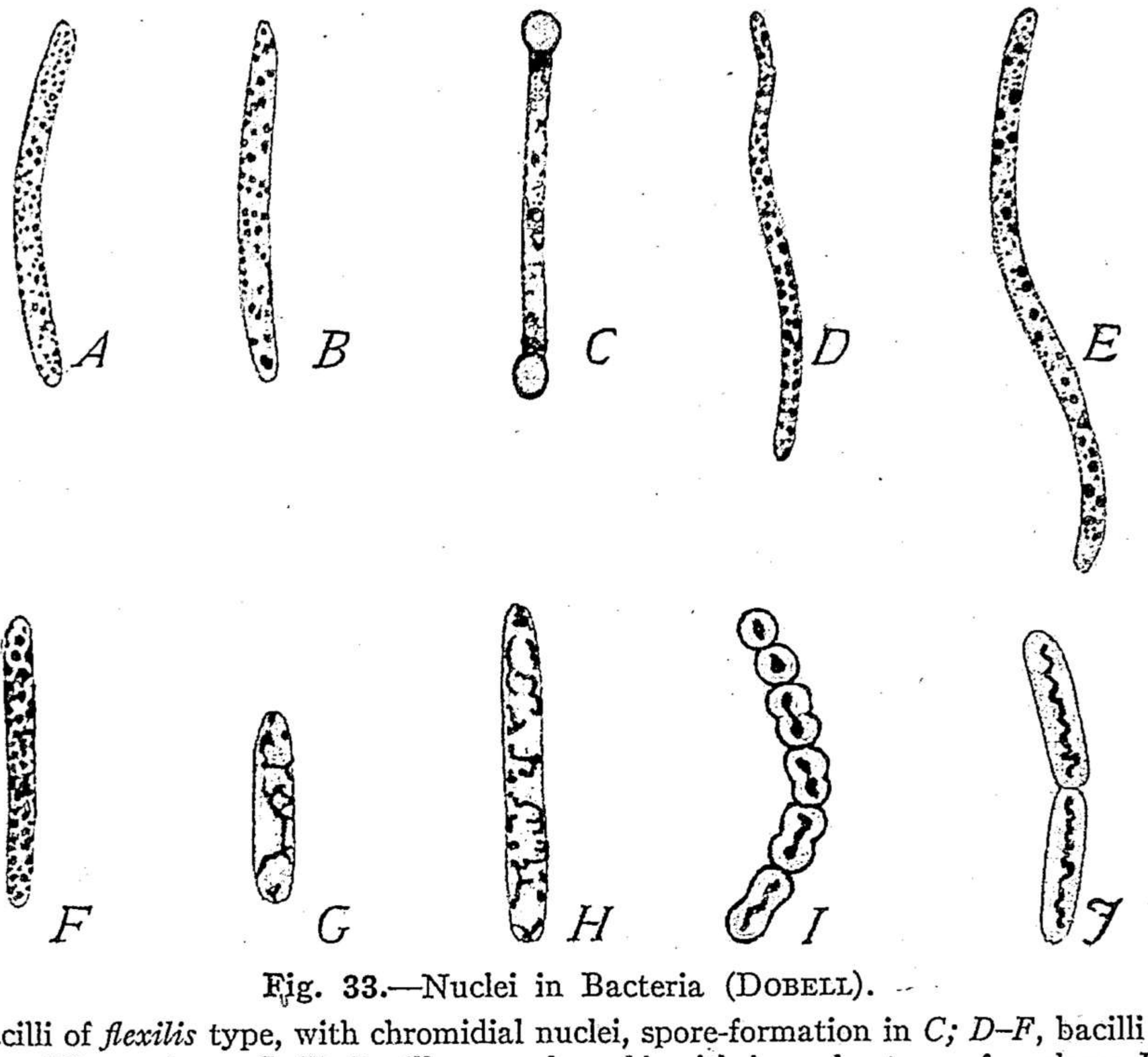

Fig. 33.—Nuclei in Bacteria (DOBELL).

A–C, bacilli of *flexilis* type, with chromidial nuclei, spore-formation in *C; D–F*, bacilli of similar type from a different host; *G*, *H*, *Bacillus saccobranchi*, with irregular type of nucleus; *I*, chain of Cocci with massive nuclei, some dividing; *J*, bacillus of *spirogyra* type, with spiral filamentary nucleus, just after division.

tive type of nucleus, though in many cases not bounded by any definite limiting membrane.[1]

B. THE NUCLEAR COMPONENTS

The vesicular nucleus, as seen in sections, usually shows four distinct components, namely: an inclosing wall or membrane; a nuclear framework usually described as a network or reticulum, though by some observers regarded as an alveolar structure; the *nuclear sap*, *enchylema*, or *ground-substance* which occupies the interstices of the framework; and one or more *nucleoli*, massive and usually rounded bodies suspended in the framework.

[1] In the Chroöcoccaceæ, a very primitive group of algæ, Acton ('14), has produced evidence that different species show intergradations between an almost undifferentiated or scattered condition of the nucleus ("metachromatic granules") and their definite aggregation to form a central nucleus, which divides into two (amitotically) prior to division of the cytosome.

1. The Nuclear Membrane

This is a delicate but usually well-defined film which often stains but slightly with cytological dyes, and sometimes can hardly be differentiated from the surrounding cytoplasm, thus resembling the wall of a vacuole; in some cases, however, it approaches the nuclear framework ("chromatin") in staining-capacity. As will be shown in Chapter II, the nuclear membrane seems in some cases to be formed from the surrounding cytoplasm, a fact which led Strasburger to regard it as analogous to the outer cell-membrane and to designate it accordingly as the "inner cell-membrane." In animal cells, however, there are cases in which the nuclear membrane seems beyond a doubt to be derived from the nucleus (chromosomes).

It has long been disputed whether the membrane is continuous or interrupted, and even whether it has any existence as a separate structure. The earliest observers considered it as a definite and continuous structure; and this view is now rather generally accepted as the correct one.[1] Somewhat later the nucleus was conceived as being only a localized area in a structural framework common to the protoplast as a whole, the nuclear membrane being no more than a denser region of the same structure; [2] and a similar view has been advocated even by some recent observers, some of whom have gone so far as to deny the existence of a nuclear membrane as a definite structure [3] regarding it as only the optical section of the peripheral zone of the nuclear framework where it comes into connection with that of the cytoplasm. The studies of Kite and Chambers on living cells by means of the micro-dissection needle seem, however, to leave no doubt of the reality of the nuclear membrane and also show that it is in some cases of very tough and resistant nature.[4]

2. The Nuclear Framework

Most of the earlier observers considered the framework (Figs. 30, 36, etc.) to be a net-like or sponge-like reticulum; and this is still the view of most cytologists. A considerable number of more recent observers, however (Haecker, Reinke, Waldeyer), have followed Bütschli in the conclusion that the framework is an alveolar structure, analogous to that so often seen in the cytoplasm, though often of different character. The mode of formation of the nucleus leads to the conclusion that both types of structure may coexist in the same nucleus; for after cell-division the framework is often produced by a process that involves not only a vacuolization of the individual chromosomes but also a formation of branches by which

[1] *Cf.* Heidenhain ('07), p. 132.
[2] See for instance, Heitzmann ('83), Van Beneden ('83–'84), Wilson ('96, '99).
[3] See Stauffacher ('10), Derschau ('11).
[4] Kite ('13), Chambers ('18b, '21b, etc.).

different chromosomes become connected to form a network (p. 135). In its earlier stages, therefore, the nucleus is a network of alveolized chromosomes (Fig. 55). In later stages it becomes difficult to distinguish between

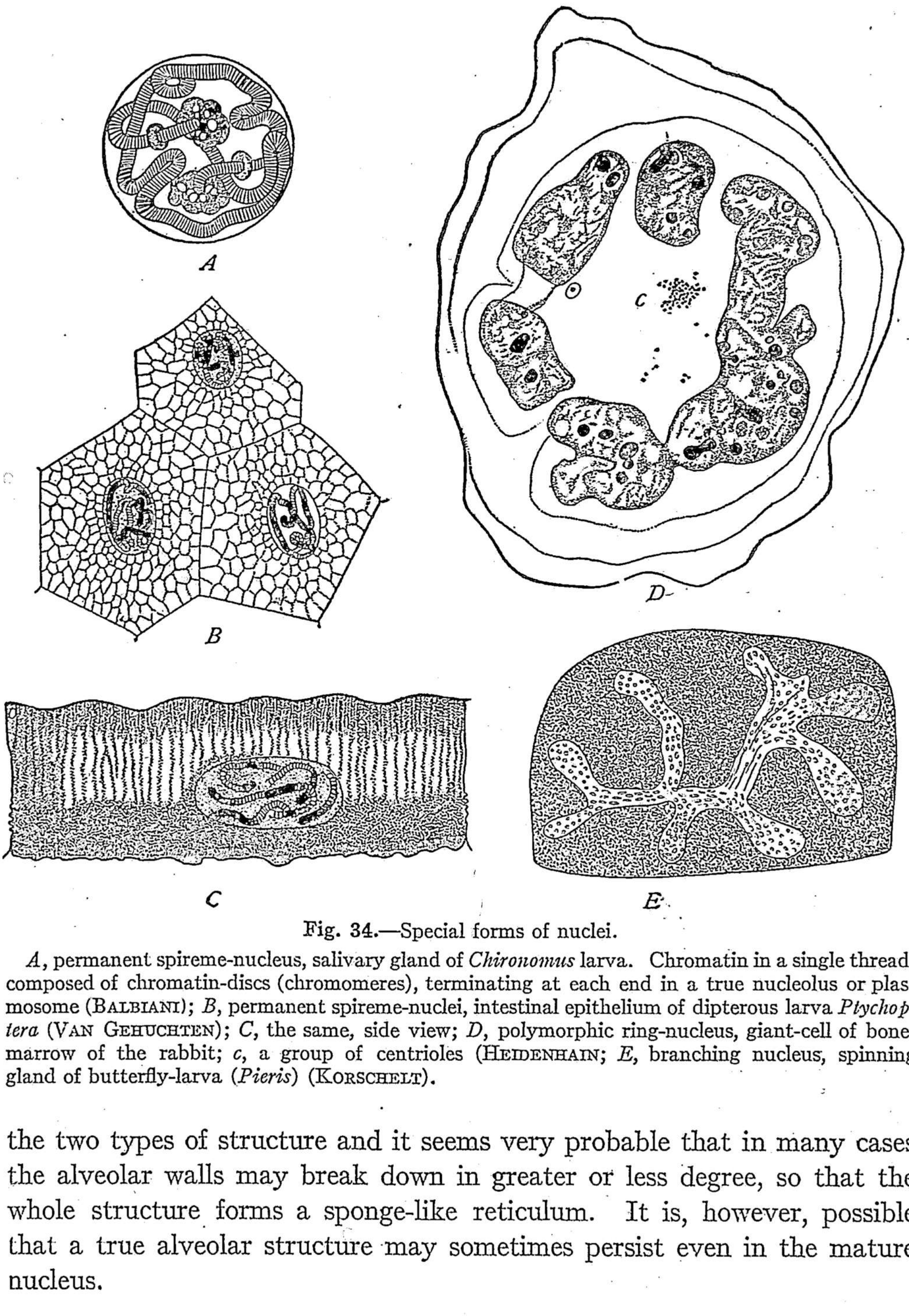

Fig. 34.—Special forms of nuclei.

A, permanent spireme-nucleus, salivary gland of *Chironomus* larva. Chromatin in a single thread, composed of chromatin-discs (chromomeres), terminating at each end in a true nucleolus or plasmosome (BALBIANI); *B*, permanent spireme-nuclei, intestinal epithelium of dipterous larva *Ptychoptera* (VAN GEHUCHTEN); *C*, the same, side view; *D*, polymorphic ring-nucleus, giant-cell of bone-marrow of the rabbit; *c*, a group of centrioles (HEIDENHAIN; *E*, branching nucleus, spinning gland of butterfly-larva (*Pieris*) (KORSCHELT).

the two types of structure and it seems very probable that in many cases the alveolar walls may break down in greater or less degree, so that the whole structure forms a sponge-like reticulum. It is, however, possible that a true alveolar structure may sometimes persist even in the mature nucleus.

The difficulties of determining this question with certainty are much increased by the fact that the character of the nuclear framework is often markedly affected by the nature of the fixatives employed.[1] When due allowance is made for this, however, it is certain that great variations in the nature of the framework exist in different kinds of cells, and the finer and closer the meshwork the greater the difficulty of determining its nature.

In fixed material, especially as viewed under relatively low magnification, the nuclei commonly appear deeply stained after treatment by certain dyes, such as carmine, hæmatoxylin, methyl-green, or gentian violet, while the cytoplasm remains relatively pale. Such dyes, accordingly, are often designated as "nuclear dyes," in contradistinction to the "plasma-dyes" which stain especially the cytoplasmic substance;[2] examples of the latter are offered by eosin, acid fuchsin, orange G or light green. It was shown by Ehrlich (1870–80) and his successors that the nuclear dyes in general, and in particular, the anilin dyes or coal-tar colors, are "basic," the plasma-dyes "acidic"[3] and it is convenient, accordingly, to designate the various cell-components as *basophilic* and *oxyphilic* according to their tendency to take up the basic or the acidic dyes. On what this tendency depends—whether on chemical affinity, on physical processes of adsorption, or on both—need not here be considered (p. 645).

The earlier cytologists, employing for the study of the nucleus mainly the basic or nuclear dyes (especially carmine, hæmatoxylin, and later safranin and gentian violet) observed that in fixed material, and after certain technical manipulation, only certain components of the nucleus were stained by these dyes. To the substance thus stained Flemming (1880) gave the name of *chromatin*, to that which stains slightly or retains the color feebly upon extraction (by acids, etc.) *achromatin*. "Chromatin," as thus defined, was considered by Flemming to be composed wholly or in part of the chemical substance "nuclein" (p. 642) and to form the more conspicuous part of the nuclear framework and also certain types of nucleoli.[4] Under the conception of "achromatin" Flemming included all the remaining nuclear substance except the enchylema. Strasburger ('82) and Carnoy ('84) recognized that the framework itself appears to consist of two constituents, namely, a continuous "achromatic" basis, and of more or less discontinuous granules or clumps of "chromatin" suspended in it (Figs. 35, 36). The first of these was found to be oxyphilic and was accordingly designated by

[1] See Lundegårdh, '12.

[2] The cytoplasm often contains various formed elements (granules, fibrillæ, etc.) that may likewise be deeply stained by the "nuclear" dyes. The term "plasma-dyes," therefore, only denotes their predominant effect on the cytoplasm considered as a whole.

[3] For further explanation of these terms see p. 646.

[4] See '82, p. 375.

Strasburger as *nucleohyaloplasm*, by Carnoy as the *plasmatic network* (composed of "*plastin*") and later by Schwarz ('87) as *linin*, a term still in common use. To the foregoing differences may be added the fact that "chromatin" as thus defined shows a high degree of resistance to hydro-

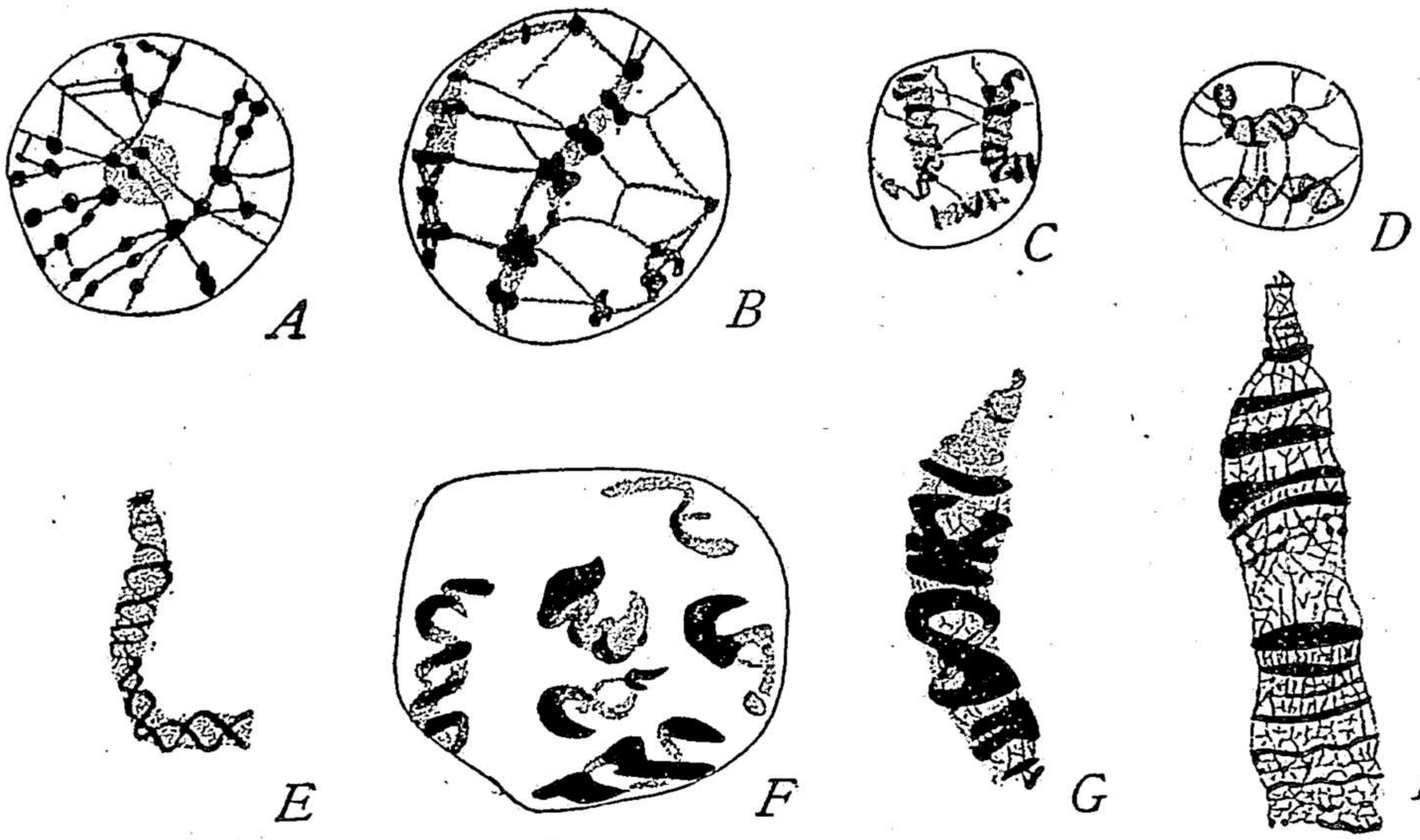

Fig. 35.—Nuclear structure in the salivary glands of larvæ of the fly *Chironomus* (ALVERDES).

A, *B*, younger nuclei, with reticulum, linin and basichromatin; *C*, *D*, *E*, origin of the basichromatic spirals; *F*, *G*, *H*, later stages, transformation of the spirals into disc-like bodies.

chloric-pepsin digestion, while the oxyphilic "linin" is less resistant in varying degree (pp. 643, 644).

Though this terminology is still in common use it involves us in many difficulties. It was found that the framework often undergoes great changes of staining-capacity in different phases of the cell-cycle and may even completely lose its affinity for the basic dyes, becoming purely oxyphilic, like linin or the general cytoplasm. Striking examples of this are offered by the egg-nucleus during the growth-period in many species of animals (p. 350); and this formerly led some observers to the illogical conclusion that the "chromatin" may completely disappear from the nucleus, and to the still more illogical inference that the nucleus, therefore, cannot be regarded as containing the basis of heredity. On the other hand, linin or "plastin" may readily be stained by acidic dyes; so that by using both a basic and an acidic dye of different colors both "chromatin" and "linin" may thus be strongly stained but in different colors. Obviously, therefore, "linin" or "plastin" is no less chromatic than chromatin. The dilemma thus arising was happily escaped by Heidenhain ('90, '07) who proposed to designate the basophilic and oxyphilic stainable nuclear materials respectively as *basichromatin* ("chromatin" of Flemming) and *oxychromatin*, con-

cluding further (in harmony with an earlier suggestion of Van Beneden's) [1] that the two substances may be only different conditions of a single substance determined by comparatively slight chemical changes—*e. g.*, by varying ratios between the percentage of nucleic acid and protein in the chromatin-substance.[2] A simple explanation is thus offered of the marked variations of staining capacity exhibited by the nuclear meshwork in different cells or in different physiological phases of the same cell; and it also escapes the supposed consequences of the disappearance of "chromatin" from the nucleus referred to above.

Many doubtful points nevertheless still remain. Heidenhain, following the lines marked out by Flemming, Strasburger and Van Beneden and other earlier observers, considers that both basichromatin and oxychromatin appear in the form of minute granules or chromioles and that both kinds of granules are suspended in a non-stainable, homogeneous substance or matrix, to which substance alone Heidenhain applies the term *linin*. The meaning of the latter term is thus greatly restricted, for it seems probable that as originally employed by Carnoy, Schwarz and their followers the "linin" or "plastin" included also much of what is now called oxychromatin. It is, however, far from certain that these granules have a persistent identity, and it is often difficult to distinguish them from mere artifacts produced by the coagulation of the reagents. We should not, however, take too sceptical an attitude towards this question, since as above stated there are cases in which granules or other formed components in the nuclear substance are clearly visible in life. One of the best of these is offered by the remarkable "spireme-nuclei" of the salivary gland-cells in Diptera long since described by Balbiani, Carnoy and other observers and more recently studied carefully by Alverdes ('12). In this case the more solid part of the nucleus appears in the form of a long convoluted thread with a nucleolus attached to each end (Figs. 34, 35). Even in the living cell this thread is seen to be composed of denser disk-like bodies suspended in a clearer basis; and in fixed preparations these bodies are found to be strongly basophilic, while the lighter substance ("linin" or "plastin") is oxyphilic.

It is extremely probable, therefore, that an analogous differentiation between basichromatin and oxychromatin exists even in nuclei where no trace of such a structure appears *in vivo*. The need of caution in this direction is, however, indicated by many facts. As every experienced cytologist knows, the character of the framework, the *apparent* number and size of the included basichromatin-masses, and the relative proportions of basophilic and oxyphilic materials, are materially affected both by the use of mordants, and by the subsequent manipulation of the dyes employed. Thoroughly con-

[1] '83–'84, p. 583, etc.

[2] See Chapter VIII, p. 652.

sistent results are indeed only reached after employment of a standard fixative and by simultaneous use of basic and acidic dyes in a mixture standardized in respect to their relative concentrations and the degree of acidification (as in the Biondi-Ehrlich mixture, p. 650). Even in this case, we are employing a method which, however carefully controlled, involves a certain arbitrarily chosen standard of performance difficult to control by other methods.

Such considerations led Lundegårdh ('10) to propose that the term "chromatin" be replaced by "karyotin" (caryotin), the substance thus designated appearing in either a basichromatic or an oxyphilic phase. This proposal has much to recommend it; nevertheless in the writer's view it is preferable to retain the older term "chromatin" provided we apply it to the whole stainable substance of the nucleus, whether basophilic or oxyphilic, and clearly recognize that basichromatin and oxychromatin are but passing phases, more or less marked and enduring, of one fundamental substance.

The physiological meaning of the changes of the nuclear framework in configuration and in staining reactions is imperfectly known. Very often

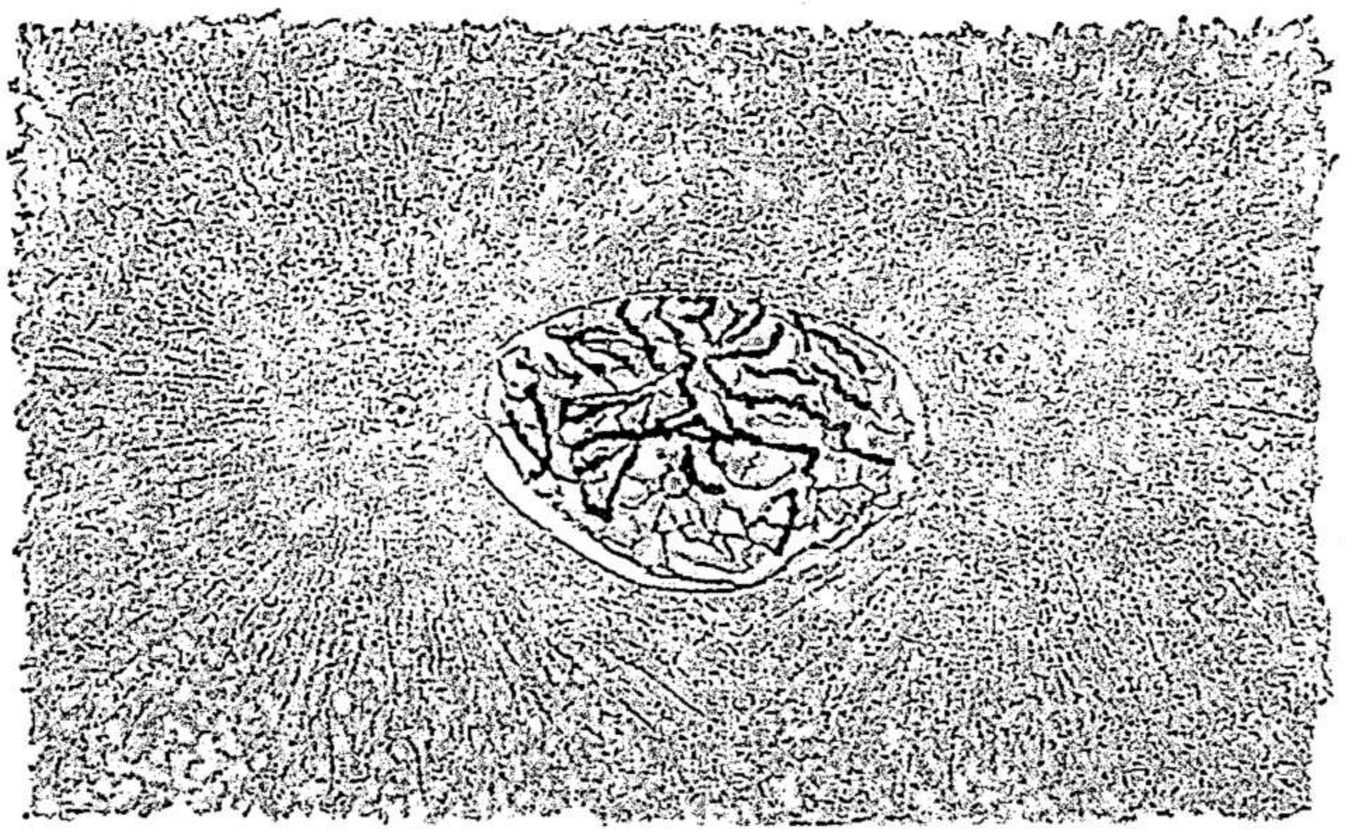

Fig. 36.—Prophase-nucleus, cleavage-blastomere of the whitefish *Coregonus*. Early chromosomes (segmented spireme), linin, central bodies and growing asters.

the nuclei of cells that are undergoing active metabolic changes, such as gland-cells or nurse-cells, contain a large amount of basophilic material and stain vigorously in basic dyes. In old and relatively passive cells, such as those of the epidermis, the reverse condition often exists. On the other hand, the fact is no less striking that in some of the most pronounced examples of actively growing cells the nuclear framework undergoes a marked diminution of its basophilic character. This appears in extreme form in the nucleus (germinal vesicle) of the egg-cell during the period of its most rapid growth in various animals (*e. g.*, in many insects, elasmobranchs and amphibians), and may even lead to a total disappearance of basophily (p. 351).

A similar diminution or loss of basophily has been observed also in the ova of plants and in the early blastomeres of the segmenting egg.

3. The Nucleoli [1]

The nucleoli are still imperfectly understood. There seem to be some forms of nuclei in which nucleoli are entirely absent; but in the nuclei of higher organisms, one or a few such bodies are almost invariably present and in extreme cases may be numbered by hundreds (p. 269). Morphologically considered the nucleoli show so many differences of form, staining-capacity and behavior as to render their classification difficult. Provisionally they may conveniently be grouped in two general classes which we shall designate as (1) *plasmosomes* or *true nucleoli*, and (2) *karyosomes* or *chromatin-nucleoli*.[2]

a. Plasmosomes. These bodies (Figs. 30, 267, 268) as their name indicates, are or tend to be oxyphilic (like the cytoplasm generally) while the karyosomes are basophilic in various degrees; but this distinction cannot be very logically carried out. In many combinations of basic and acid dyes, for example safranin and light-green or hæmatoxylin and eosin, the plasmosomes are sharply stained by the acidic dye; but in Flemming's triple mixture (safranin-gentian-orange) they stain characteristically with the basic safranin, though less intensely than the chromosomes; furthermore, their staining reaction is often markedly affected by the mode of fixation. Beyond this, the staining-reactions of these nucleoli often vary materially at different periods in the history of the nucleus; so that the same nucleolus may be at one time oxyphilic and at another time basophilic. It thus becomes probable that the varying staining reactions of the nucleoli are to a certain extent analogous to those of the chromatin of the nuclear framework, and may likewise be due to corresponding variations in chemical conditions. Such considerations formerly led to the view [3] that the nucleoli consist essentially of a basis of oxyphilic "plastin" or the like ("pyrenin" of Schwarz) which when impregnated with a basophilic "chromatin" becomes a basophilic or chromatin-nucleolus, and that the varying conditions of staining-reaction and digestibility are due to varying proportions and distribution of these two components. It is, however, more in accordance with present conceptions concerning the relations between basichromatin and oxychromatin to think of these varying reactions as due to different phases of a single original substance which may assume the

[1] For an exhaustive review of the earlier literature of this subject, see Montgomery, '98; of the later, Ludford, '22.

[2] This distinction is based on that of Flemming ('82) who designated these respective classes as "true nucleoli" (or simply "nucleoli") and "net-knots." The terms plasmosome and karyosome are due to Ogata ('83).

[3] R. Hertwig ('98), Farmer ('07), Reed ('14), etc.

basophilic or the oxyphilic condition according to changes in its chemical composition, *e. g.*, to varying ratios between the nucleic acid and the protein components (p. 652). This would equally well explain the fact that in general the oxyphilic nucleoli are readily attacked by pepsin-hydrochloric (Zacharias) while basophilic nucleoli (or nuclear components) are more resistant in various degrees (Jörgenssen, '13, etc.); but there are conspicuous exceptions to this—*e. g.*, the peripheral nucleoli of Amphibia (*Salamandra*).[1]

It is clear from the foregoing that it is often difficult to identify the plasmosomes by their micro-chemical reactions alone, though in "typical"

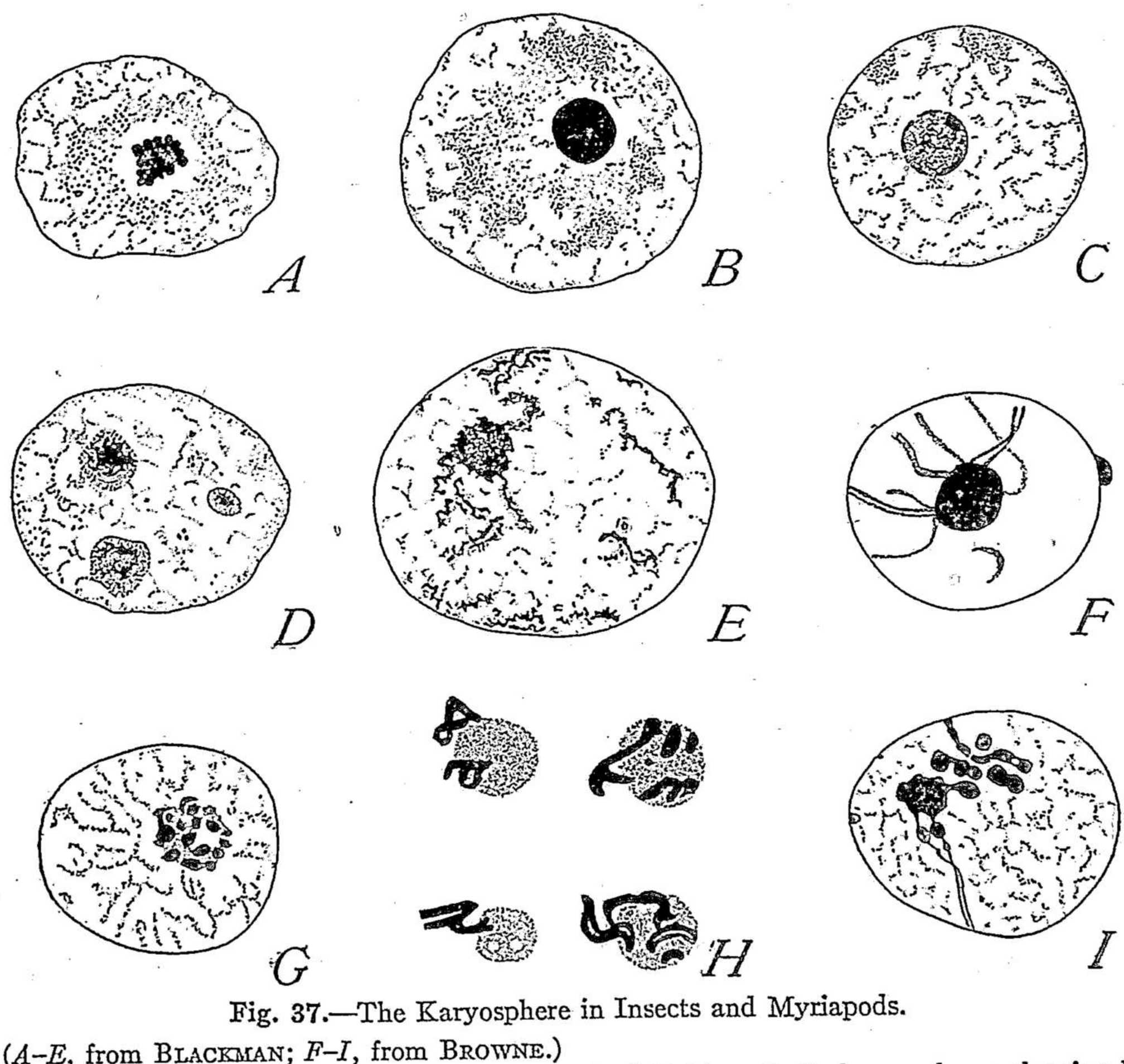

Fig. 37.—The Karyosphere in Insects and Myriapods.

(*A–E*, from BLACKMAN; *F–I*, from BROWNE.)

A, earlier and *B*, later stage of spermatocytes in *Lithobius; C*, *D*, karyospheres showing both basichromatin and nucleolar substance; *E*, escape of chromosomes from karyosphere, *Scolopendra; F*, early spermatocyte of the hemipter *Notonecta; G*, later stage; *H*, escape of chromosomes leaving plasmosome-remnant; *I*, formation of the chromosomes.

cases they are readily recognizable by the use of double stains. In a broader sense their identity can only be fully established *by their morphological history;* for these nucleoli do not contribute directly (*i. e.*, as formed elements) to the formation of the chromosomes (p. 141). For the rest, it

[1] See p. 270.

may be said that the plasmosomes most commonly appear as sharply defined, rounded and rather highly refractive bodies, not directly connected with the general framework of the nucleus in which they are suspended. Physically they appear like liquid drops; and that they are at least semi-liquid is indicated by the forms which they assume when flattened out against the nuclear membrane, or against other nucleoli, both of which conditions are sometimes seen in the nuclei of the auxocytes in both sexes. Sometimes they are of irregular shape and undergo active changes of form in living cells.[1] As a rule they are devoid of a distinct limiting membrane; though in some cases they are surrounded by a basophilic envelope.

b. The karyosomes (Ogata), or chromatin-nucleoli (Montgomery). The bodies thus called are intensely basophilic, like basichromatin, and show the same high degree of resistance to peptic-hydrochloric digestion. They contrast sharply with the true nucleoli or plasmosomes in the fact that they contribute directly to the formation of the chromosomes, during cell-division. They are of at least three well-marked types, as follows:

Net-knots, as originally distinguished by Flemming, of more or less irregular form, often variable in size and number, and typically in direct continuity with the nuclear framework, of which they seem to be no more than thickened nodes (Fig. 8). They differ only in degree from the small granules or clumps of basichromatin, and like the latter give up their substance to the spireme-threads and chromosomes in the early stages of mitosis (p. 141). A transition to chromatin-nucleoli of more definite type is, however, given by the "prochromosomes" which are of similar general type, but are of constant number, equal to that of the chromosomes (Overton, Rosenberg, etc.) and are believed by many good observers to be converted directly into chromosomes or at least to serve as centers for their formation (p. 901).

Chromosome-nucleoli, known with certainty only in the nuclei of the gamete-producing cells (auxocytes and sometimes in the gonia, p. 759). These are sharply defined, usually spheroidal, and not continuous with the general framework. They represent either single chromosomes, or a small group of chromosomes, which persist in a condensed and rounded form during the "resting" or vegetative phase of the nucleus. They are best known in case of the sex-chromosomes, which are in general characterized by this behavior during the growth-period of the spermatocytes in many animals (Figs. 266, 267).

Karyospheres. These nucleoli, equivalent to the "*nucléoles-noyaux*" of Carnoy, are spheroidal bodies (Figs. 37, 109) commonly of large size

[1] Balbiani ('64); see Montgomery ('98).

which at certain stages contain all, or nearly all, the basichromatin in the nucleus (*e. g.*, the nucleolus of *Spirogyra*, or that of the spermatocytes of certain insects and myriapods) and from them arise the entire group of chromosomes (or many of them) in mitosis. Among the Protozoa, especially in rhizopods, the nucleus often contains a very large body of this type, commonly called by protozoölogists the "karyosome," which is described in some cases as giving rise to all of the chromosomes in mitosis, in others to only a part of them. In these cases the karyosome plays the part also of a central body or division-center, thus giving a possible transition to intra-nuclear central bodies (p. 204). For this reason, among others, it seems doubtful whether these bodies are closely comparable to the karyosomes of multicellular forms.

c. Amphinucleoli. Plasmosomes and karyosomes frequently coexist in the same nucleus, sometimes quite separate, in other cases closely associated to form a double nucleolus or *amphinucleolus*; the latter are commonly seen in the eggs of various mollusks, annelids and arthropods (Fig. 108), and in the spermatocytes of insects. In many of these cases the chromosome-nucleolus is often in its earlier stages attached to a plasmosome, though afterwards separating from it (Fig. 267). In some cases one or more chromosome-nucleoli are imbedded in a large plasmosome; in others all the chromosomes, in the form of closely crowded chromosome-nuclei, appear to be imbedded in a plasmosome to form a karyosphere (Fig. 37); and it is possible that all karyospheres are of this nature.

The origin of the nucleoli is still to a considerable extent in doubt; but the evidence is accumulating that all forms of them may be directly derived from the chromosomes. This is obviously the case with the various forms of chromatin-nucleoli, the origin of which has in many cases been traced step by step, especially in the case of chromosome-nucleoli (p. 759). In case of the plasmosomes the facts are not so evident; but here, too, there is reason to conclude that these nucleoli may arise by a direct transformation of a portion of the chromatin thread.[1] The questions here involved are hardly separable from those next to be considered.

d. Functions of the Nucleoli. The physiological meaning of the nucleoli still remains one of the most obscure questions of cytology. In case of the chromatin-nucleoli or karyosomes no great difficulties are encountered. In one form or another they are localized reservoirs of basichromatin, though we do not know why they should assume a compact form in an apparently inactive condition. Concerning the true nucleoli or plasmosomes we are still for the most part confined to indirect evidence and conjecture. In the later prophases of mitosis these nucleoli most commonly

[1] See Carothers ('13), Wenrich ('16, '17).

disappear, often previously becoming reduced in size or under going fragmentation. In some cases they are cast out bodily into the cytosome at the time the nuclear membrane disappears and there sooner or later degenerate, though sometimes persisting for a long time.[1] From this fact arose the view of Haecker, later held by many others, that the plasmosomes are accumulations of waste-products or by-products of the nuclear action, derived from the chromatin either by direct transformation of its substance, or as chemical cleavage-products or secretions (nuclear secretion-hypothesis). On the other hand, many cytologists, from the time of Flemming ('82, '91) have considered the nucleoli generally as centers for the storage or elaboration of substances such as "linin," "plastin" or "chromatin," destined to play some definite part in the later operations of the nucleus ("transportation-hypothesis of Haecker").[2]

This view is obviously correct as applied to the karyosomes. Flemming regarded the plasmosomes, likewise, as somehow concerned in the storage of "nuclein" or chromatin, or of materials necessary for the production of these substances. Strasburger ('98, '94, etc.), regarded the true nucleoli as storehouses of "kinoplasm," or material from which the spindle-fibers are formed during mitosis. There is, however, little definite evidence in support of this, while it is opposed by the fact, later to be described, that in animal cells perfect spindles and asters may be formed, one after another in regular succession, in the entire absence of nuclei (p. 176). More serious attention is demanded by the fact, especially striking during the growth-period of the oöcytes of many animals, that at the period when the chromosomes and the nuclear framework have become completely oxyphilic the nucleoli (which appear to be morphologically plasmosomes) are often intensely basophilic (pp. 270, 353).[3] This suggests that the nucleoli may in such cases be storehouses of nucleic acid to be drawn upon at a later period when the chromosomes are resuming their basophilic character. This, however, is purely hypothetical.

Observations have begun to accumulate in favor of the conclusion that the true nucleoli may be concerned in the secretory processes of the cell. Many earlier observers described a discharge of nucleolar fragments, or even of entire nucleoli, into the cytosome; and more recently a number of observers have found that such nucleolar material may give rise to various kinds of secretory products or storage-bodies. A conspicuous case of this is offered by the formation of yolk-spheres in oögenesis (p. 345). In the

[1] See Haecker (92, '93), Karsten ('93), Wheeler ('97), etc.

[2] See, for instance, Flemming ('82, '91), Went ('87), O. Hertwig ('93), Rhumbler ('93, '00), Carnoy and Le Brun ('97, '98), Lubosch ('02). For general and critical reviews see Haecker ('95, '99) Strasburger ('95, etc.), Montgomery ('99), Nemec ('10), Jörgenssen ('13), Buchner ('18).

[3] On this point see especially Jörgenssen ('13), also Maréchal ('06).

tissue-cells the extrusion of nucleolar fragments has been described by many observers,[1] several of whom believe they have traced to this source the origin of various formed bodies in the cytoplasm such as fat-drops and mucin-bodies (Schreiner, '15, '16) albuminous granules (Nakahara, '17, '18) and other products. To the writer none of these cases yet seems to be satisfactorily demonstrated, and the question is a most difficult one to be settled by studies on fixed material alone. Until the facts have been decisively demonstrated by the study of living cells judgment on these cases should be suspended. Nevertheless the observations in question prominently raise the question whether the nucleolus may not play a more active and important part in cell-metabolism than most writers have hitherto assumed.

4. The Enchylema, Ground-substance or Nuclear Sap

This has commonly been regarded as a structureless and non-stainable liquid, but the studies of Kite ('13) and Chambers ('14, etc.) show that this substance is in some cases of much firmer consistency than was formerly supposed. Heidenhain's important studies, already referred to, have shown that the spaces occupied by the enchylema may be much more restricted than appears after staining by a single "nuclear" or basic dye; for upon staining also with an acidic dye the spaces are often found to be occupied in greater or less degree by oxychromatin granules, and the meshwork thus appears to be correspondingly extended at the expense of the enchylema. The material thus brought into view is, however, often readily seen without use of the acidic dyes. It is very difficult, perhaps impossible, to determine how far these granules preëxist in life and how far are only coagulation-products of the enchylema. Zacharias ('02, etc.) has shown in various plant-cells, that in the early prophases of division, when the chromosomes are visible in fresh cells, a granular or net-like substance is immediately brought into view upon treatment by alcohol, HCl, and other coagulating agents. This material is dissolved by peptic digestion, while the basichromatin remains undigested. It seems probable that this material is in part thrown down from the enchylema or ground-substance; but it perhaps corresponds in part also to the linin and oxychromatin in Heidenhain's sense. This subject calls for further elucidation.

5. Other Structures

The nucleus may contain still other formed elements of less constant occurrence, of which the best known is the intra-nuclear division-center,

[1] See Lukjanow ('87), Montgomery ('98), Walker and Embleton ('08), Walker and Tozer ('09), etc.

or "nucleolo-centrosome," which plays the part of a central body during mitosis. This is very rare among higher forms; a classical case is the intra-nuclear center of the spermatocytes in *Ascaris megalocephala univalens*, discovered by Brauer (Fig. 323). In Protozoa such intra-nuclear centers are of common occurrence in flagellates (Fig. 88) and rhizopods (Fig. 87). The relation of these bodies to the extra-nuclear centers and to the blepharoblasts will be considered elsewhere (p. 690).

Other and less familiar intra-nucleolar bodies include small deeply staining granules or *nucleolini*, which have been described by many observers.[1] Carleton ('20) has recently produced evidence that certain types of these bodies may divide regularly in mitosis, the products being distributed regularly to the daughter-nuclei, while the nucleoli themselves disintegrate. Possibly, therefore, they may serve as centers for formation of the nucleoli; but nothing is known of their significance. Besides the foregoing may be mentioned intra-nuclear rodlets or straight axial fibrillæ (distinct from the nuclear framework) that have been found in the nuclei of some kinds of sperm-cells by Retzius, Champy and others and also in the red corpuscles of birds.[2] They are of unknown significance.

V. QUANTITATIVE RELATIONS OF NUCLEI, CELLS AND CELL-AGGREGATES

1. Cell-size and Body-size

All cells are subject to considerable fluctuations of size; nevertheless, within rather wide limits of variability the size of cells, like that of the body they may build, may be regarded as a specific constant. This is true alike of multicellular and unicellular organisms. An interesting example of the latter case is offered by the ciliate *Paramœcium caudatum*, a species in which Jennings ('08, '11) found it possible to isolate at least eight different races or strains which differ characteristically in size and breed true. Though each of these races is subject to considerable fluctuation the mean or norm is constant, the largest form being about five times the length of the smallest (Fig. 38). The smallest visible cells (Fig. 39) probably occur among the bacteria, some of which (*Cocci*) lie almost at the limit of microscopical vision; but it is not impossible that still smaller cells exist, that cannot be seen even with the highest powers of the microscope. At the other extreme, so far as linear dimensions go, are probably those nerve-cells and their branches which innervate the extremities of the large mammals. Such neurons may attain a length of several feet. In volume, the upper existing

[1] See Montgomery ('98b).

[2] See Champy and Carleton ('21).

limit of magnitude is probably attained by the huge eggs of certain birds and sharks, and some of the extinct forms are known to have been larger

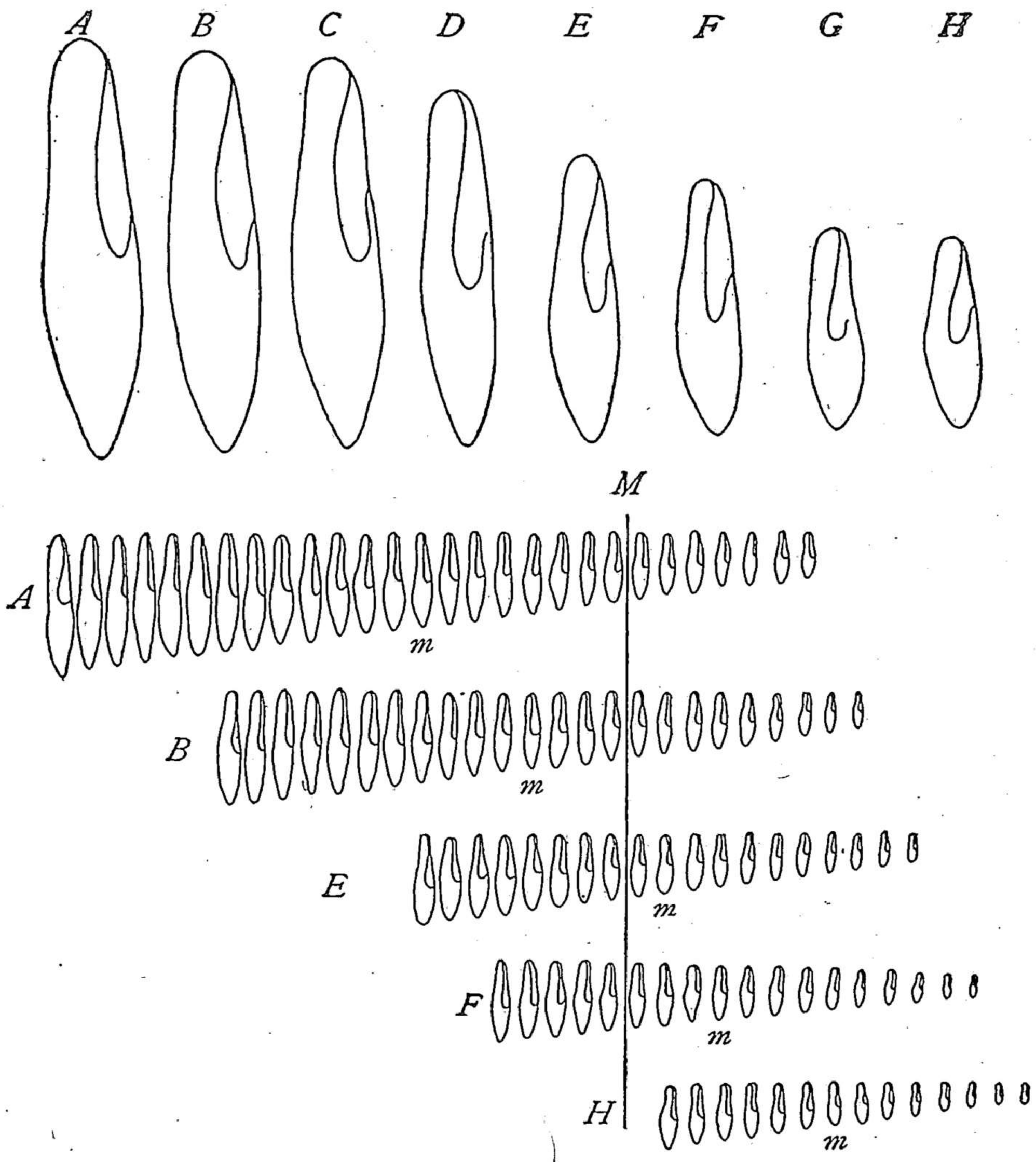

Fig. 38.—Eight races of *Paramœcium* (JENNINGS).

Upper row, diagram showing the relative mean size of races A–H. Below, diagrams of races *A*, *B*, *E*, *F*, and *H* (less highly magnified), to show range of fluctuation within each race. The mean size of each race indicated at *m*, that of the whole eight races indicated by vertical line *M*.

still. Among modern birds the largest egg-cell is that of the ostrich. Externally this egg is in round numbers about 6 inches in length, while the yolk (which alone represents the egg-cell) has a mean diameter of about 80 mm. or a little more than 3 inches.[1] The egg-shell of the extinct giant

[1] This is my own measurement of a fresh, unincubated egg. Measurements of the egg of the great shark, *Chlamydoselache*, from an alcoholic specimen in the Columbia museum, give a slightly higher value. For photographs, side by side, of the eggs of the hen, the ostrich, the extinct moa of New Zealand, and *Æpyornis*, see Lucas, *Animals of the Past*, Fig. 20.

bird *Æpyornis*, from New Zealand, measures about 13 inches in length; from which (assuming yolk and shell to have had the same relative proportions as in the ostrich) the diameter of the yolk should have been approximately 7 inches. If we estimate the diameter of the ostrich egg-cell (yolk) as 75 mm. and that of the smallest visible *Coccus* as .001 m. the ratio of their linear dimensions is as 75,000:1 and that of their volumes as $(75,000)^3$:1.

Such a difference is of the same order as that between a sphere of one inch in diameter and one of more than a mile, or between a sphere 500 feet in diameter and the earth.

The size of cells is to a certain extent characteristic of larger groups; for instance, amphibians in general have much larger cells than reptiles, birds or mammals; gymnosperms larger cells than angiosperms, and monocotyledonous plants larger ones than dicotyledonous. In a measure these differences are correlated with the rate of activity, so that it is almost proverbial among cytologists that relatively sluggish and clumsy animals, such as Orthoptera or urodeles are more likely to afford large and favorable cells for study than active ones such as Hymenoptera, Diptera or birds.

Like the size of cells, the size of the multicellular body is, within a certain range of variation, a specific constant, and in some cases follows the laws of Mendelian heredity, as shown by Mendel's familiar experiments on short and tall races of peas. The factors by which body-size is determined are of at least three widely different types.

(1) In a large class of cases, including both plants and animals, it has been demonstrated that within the species individuals of different size do not differ noticeably in respect to the size of their constituent cells, but only in respect to their number. This was first determined in plants by Amelung ('93) and by Strasburger ('93). Rabl ('99) found the cells of the crystalline lens to be nearly constant in size but variable in number, the size of the lens varying accordingly. Boveri ('04) found that epithelial cells and bone corpuscles from human dwarfs and giants are of the same size as in normal individuals. Conklin ('96, '12, '13), in an extended study of snails of the genus *Crepidula*, found a similar relation between different species. The size of an average male of *C. fornicata* is about 125 times that of an average male of *C. convexa;* in *C. plana* the size of an average female

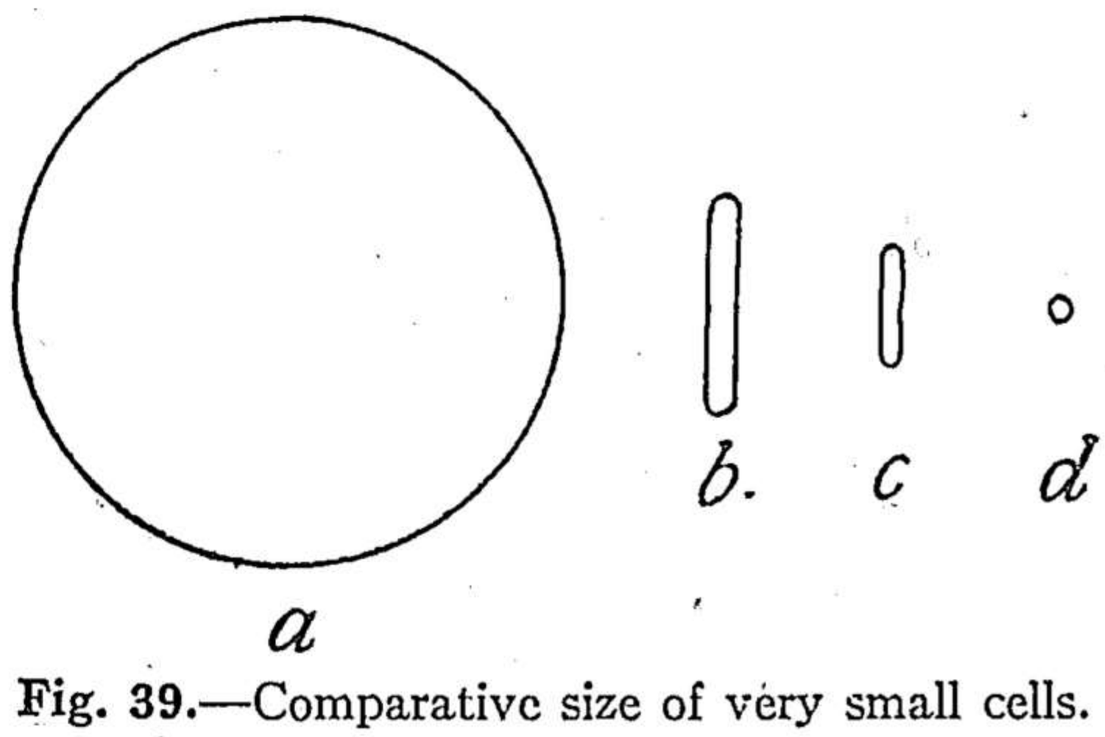

Fig. 39.—Comparative size of very small cells.

a, human erythrocyte or red blood-corpuscle (6 μ); *b*, typhoid bacillus (2.4 × 0.5 μ); *c*, influenza bacillus (0.5 × 0.2 μ); *d*, germ of poliomyelitis of Flexner and Noguchi (0.15–0.3 μ). (From Jordan's *General Bacteriology*, excepting *d*).

is about 15 times that of a dwarf female. In all these the size of the tissue-cells is in general nearly the same,[1] and the great differences of body-size are wholly due to variations in the number of cells. This does not apply to the sex-cells (ova) of different species, which differ widely in size. Within the same species, however, the ova are nearly of the same size but differ in number in individuals of different size, just as in case of the tissue-cells.

(2) The foregoing cases include only indefinite variations or fluctuations within the species. A second and quite different kind of giantism results from an increase in the size of cells without corresponding increase in their number. Typical of this class are certain of the so-called *gigas* races or species of such plants as *Œnothera* or *Primula*, in which the cells are distinctly larger than those of the normal types, though with a considerable range of variation. In the most typical cases this involves a corresponding increase of body-size, though sometimes this is seen only in certain parts.[2] In giants of this type the nuclei are correspondingly increased in size, and in most cases are tetraploid, *i. e.*, divide with twice the usual or diploid number of chromosomes (p. 728). It has been proved experimentally that the increased cell-size in certain of these cases is due to the increased nuclear size, which in turn is due to the doubled number of chromosomes. A classical case is offered by the experimental results of Gerassimoff ('02) on the fresh-water alga, *Spirogyra*. By exposing the normal forms to lowered temperature, and in certain other ways, it was found that mitotic division may be so modified that although the chromosomes divide the daughter-nuclei do not separate normally and cytoplasmic division fails. Binucleate cells are thus produced, the two nuclei either remaining separate or fusing into one, which then grows to twice the normal size. In either of these cases the doubling of the nuclear mass is followed by growth of the cytosome to double the normal volume; and by the continued division of such cells are produced giant filaments which may be reared to maturity, produce gametes of double the normal size, and conjugate to produce correspondingly enlarged zygotes (Fig. 313).

In this case the number of chromosomes is not certainly known; but there can be no doubt that it is doubled, so that the giant races may be called tetraploid. Certain tetraploid giant forms of *Œnothera*, *Primula* and *Solanum* (p. 728) are known to have arisen as sudden mutations from species of normal size and diploid chromosome-number. It is practically certain that they have been produced by a process of similar type; and this is known to be the case also in tetraploid mosses and sea-urchins experimentally produced by the Marchals and by Boveri (p. 729).

(3) In the foregoing cases, the chromosomes of the tetraploid forms, so

[1] Ganglion-cells and muscle-cells are said to form an exception.

[2] See p. 731.

far as they have been examined, appear to be of the same size as in the normal or diploid forms; and since their number has been doubled the total mass of "chromatin" is also double the normal. In a third class may be included cases of giantism which cannot be included in either of the first two, the most striking of which are *gigas*-forms having the normal or diploid chromosome-number. Examples of these are certain mutants of *Primula sinensis* (Gregory,'09), and of *Œnothera Lamarckiana* (Stomps, '16, '19), both belonging to genera in which tetraploid *gigas*-forms also are known; and similar diploid giants were found by Stomps in *Narcissus*. Beside these cases may be placed the curious one of *Primula kewensis*, a tetraploid mutant of hybrid origin, which is tetraploid and has larger cells and nuclei than the parent forms, but in which the chromosomes are but half the typical size. In this case increased size of nuclei and cells (in the approximate ratio 5:4) seems to have occurred without any increase in chromatin-mass (Farmer and Digby, '07).

From all this it is clear that the quantitative relations of chromosomes, nuclei, cytosomes and cell-aggregates offer a complex problem, and one that is incompletely solved. Nevertheless the undoubted causal relation between nuclear volume and cytoplasmic growth (*i. e.*, the *karyoplasmic ratio* of R. Hertwig) is a fact of great theoretical interest.[1]

VI. THE CELL IN RELATION TO THE MULTICELLULAR BODY

The body, we are accustomed to say, is built up of cells or their products (p. 3). In what sense do we use this phrase, and what is the morphological and physiological relation of the cells to the body which they form? These questions first arose with Schwann, who offered an admirably lucid discussion of the facts so far as known to him (1840). It was his conclusion that the cell should be regarded as a primary organic unit or elementary organism. The life of the higher organism, in his view, is essentially a composite. Each cell has its independent existence or individuality; and "the whole organism subsists only by means of the reciprocal action of the single elementary parts." [2] This conclusion took on new significance with the conclusion of Siebold (1845) that in the Protista or lowest forms of life the whole body consists of but a single cell; for this suggested the view that the multicellular body of higher forms is equivalent to an assemblage or colony of one-celled individuals; and from this grew the further conception that the multicellular organism may be regarded as a "cell-state" the one-celled members of which have undergone a physiological division of labor.[3]

[1] For further discussion see p. 727.

[2] *Untersuchungen*, Eng. Trans., Sydenham Soc., p. 181.

[3] A considerable group of modern authorities have sought the origin of Metazoa in syncytial or multinucleate rather than actually colonial forms (Jhering, A. Sedgwick, Delage).

Elaborated especially by Milne-Edwards, Virchow and Haeckel, this conclusion offered a simple and natural point of attack for the problems of cytology, embryology, and physiology, and revolutionized the problems of organic individuality. Its value as a means of biological analysis needs no other demonstration than the immense advances that it made possible. Inevitably in practice we treat cells as distinct, though closely coördinated, elementary organisms or organic units; and although some writers have questioned the validity of this procedure (p. 103) it nevertheless remains an indispensable means of analysis.

That cells are elementary organisms, having a high degree of independence, is an obvious fact in case of the Protista and of the germ-cells of all higher organisms. It is hardly less obvious in case of the blood-corpuscles, the wandering leucocytes and other separate cells in the multinuclear body. It is certain also, as will later be shown (p. 1031), that in certain of the lower multicellular types, including even such forms as sponges, hydroids and polyps, a highly differentiated multicellular body may be built up by the aggregation of cells previously more or less completely separate. Further, it has been shown by Harrison, Burrows, M. R. and W. Lewis, and others that small groups of muscle-cells, epithelia, connective-tissue-cells, embryonic nerve-cells and others, may be removed from the body and kept alive in suitable cultivation media *in vitro*, where they may continue to grow and multiply for long periods, in some cases for several years (p. 234) without loss of their specific character. Again, it has long been known that in some of the higher plants, such as *Marchantia*, *Begonia*, or *Torenia*, a very small fragment of the body, perhaps even a single cell, may give rise to a complete plant.

All this tends to support the conclusion that fundamentally the cell possesses in itself the complete apparatus of life, and to this extent tends to sustain Schwann's general conception. On the other hand, it is obvious that under normal conditions the physiological autonomy of the tissue-cells is in considerable degree merged into the life of the organism considered as a whole. This is due to a process of integration and differentiation through which the tissue-cell often comes to appear as no more than a localized area of specific activity, provided it is true with the complete apparatus of cell-life and even capable of independent action within certain limits, but still remaining a part and not a whole. This conclusion is most clearly brought out by the phenomena of growth and development, which seem to show that the multicellular body arises by the splitting up of a unicellular germ without impairment of the individuality of the organism as a whole (p. 1029). From this point of view the apparently composite character of the individual may be conceived as due to a secondary distribution of its energies among

localized centers of action. This, however, is not subversive (as some writers have assumed) of our fundamental conception of the cell-state. It is paralleled by the integration and division of labor seen in such organisms as the Pennatulaceæ (*e. g.*, *Renilla*) or the Siphonophora which are undoubtedly colonies of simpler individuals yet display a high degree of individuality considered as wholes. We shall therefore proceed upon the assumption, if only as a practical method, that the multicellular organism in general is comparable, to an assemblage of Protista which have undergone a high degree of integration and differentiation so as to constitute essentially a cell-state.[1]

From any point of view the physiological and structural interrelations of the tissue-cells remains a fundamentally important question. Apart

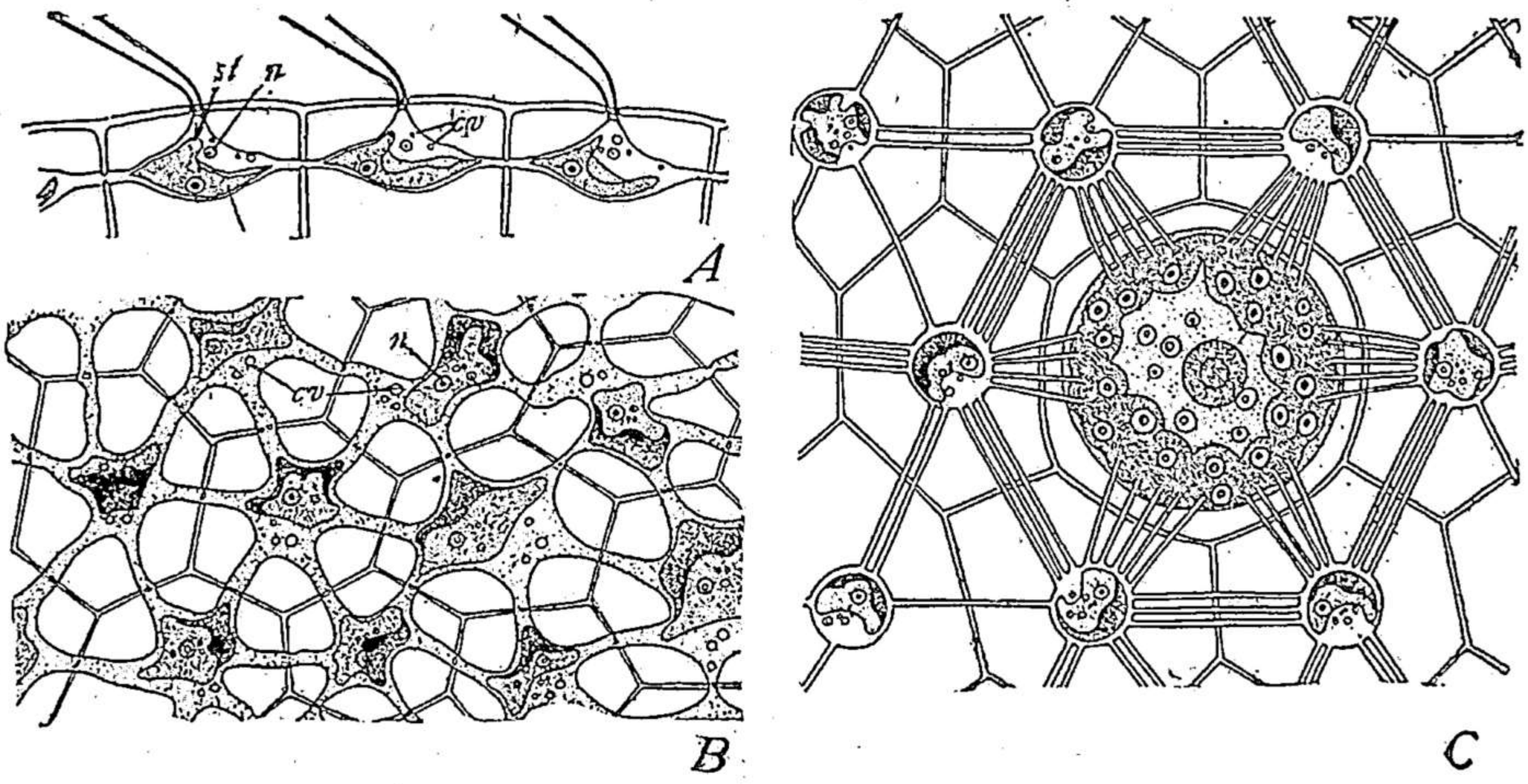

Fig. 40.—Protoplasmic cell-connections (plasmodesms), in *Volvox*, somewhat schematized (JANET).

A, *V. globator*, in vertical optical section; *B*, in surface view, showing broad bridges; *C*, *V. aureus*, gonidium, connected with surrounding vegetative cells by fine bridges: *cp.*, chloroplast, *cv*, contractile vacuoles; *p*, pyrenoid, *st*, stigma.

from the nervous mechanism and that provided by the soluble enzymes, hormones and other chemical substances,[2] it is probable that an important part in the coördination of the cell-activities is played by direct protoplasmic connections between cells ("cell-bridges," "plasmodesms"). Heitzmann long since (1873) held that even when distinct cell-walls are formed they are still traversed by strands of protoplasm by means of which the

[1] This view has been vigorously assailed by many writers, especially by those who have emphasized the conception of the "organism as a whole." See, for instance, Whitman ('88, '93), A. Sedgwick ('94), Dobell ('11), Child ('15) and especially Ritter ('19). Such criticisms seem to ignore the probable historical origin of multicellular from unicellular organisms, as well as the fundamental general similarity between the protistan cell and that of the metazoön or metaphyte, both in structure and mode of origin.

[2] See Cunningham ('21), Adami ('17), etc.

protoplasts remain in protoplasmic continuity. The whole body was thus conceived by him as a more or less continuous mass, the cells being no more than nodal points in a general network of protoplasm. This interesting conception, at first received with extreme scepticism, has met with considerable support from later observation. Direct protoplasmic cell-connections have long been known in colonial Protista, and in various simple algæ and fungi. A striking example is seen in *Volvox*, where the small somatic cells are connected both with one another and with the gonidia or germ-cells (Fig. 40); and more or less similar cell-connections are often seen in colonial flagellates, ciliates and other Protozoa. In multicellular organisms cell-bridges have been demonstrated in many forms. Their existence in the sieve-tubes of higher plants has long been known, and the researches of Tangl, Gardiner, Kienitz-Gerloff and their successors demonstrated their existence in many other tissues.[1] In lower plants, the protoplasmic bridges may be either broader strands (*e. g.*, in *Volvex globator*, and in red algæ) or fine filaments (*V. aureus*). In higher plants they are typically very fine and delicate fibrils, often invisible until after suitable staining. Cell-bridges of this type may be solitary, or scattered, or grouped together in bundles at the bottom of pits in the cell-wall where they pierce the pit-membrane or middle lamella of the wall.

In animal tissues the existence of both cell-anastomoses and of intercellular bridges is now well established for many kinds of cells. In certain forms of connective tissue-cells and cartilage-cells, also the bone-corpuscles, the scattered cells are often connected by anastomoses to form more or less net-like very delicate strands traversing the inter-cellular substance.

Plasmodesms or cell-bridges are of general occurrence in the epithelial tissues, where they were first observed in epidermal "spine-cells" ("Stachelzellen") and supposed to be spine-like processes from the membrane or cell-periphery (M. Schultze, 1864). Later studies by many observers, (Ranvier, Renaut, Pfitzner, Schridde, Kromayer, Cajal, etc.) proved these structures to be protoplasmic inter-cellular bridges, and further showed that they are traversed by fibrillæ, which may be followed from one cell to another and even through several cells (Fig. 41). The plasma-bridges have since been found in the columnar epithelia generally.[2] Further, it has been shown by a considerable number of observers that the germ-cells in both animals and plants may be connected with the surrounding somatic cells (follicle

[1] Tangl ('79–'81), Gardiner '88, (98, '00), Keinitz-Gerloff ('91,'02), A. Meyer (96, '02), Kühle ('00), etc. Critical reviews with literature, in Kienitz-Gerloff, Strasburger ('01), and Davis ('05). See also Hill ('00, '01).

[2] Literature in Flemming ('95, '97), Heidenhain ('07, '11), Studnicka ('98, '09, '13), O. Hertwig ('12). It is probable that the plasma-bridges described in smooth muscle-cells belong to the interstitial connective tissue and may be shrinkage-products (Heidenhain).

cells, etc.) by protoplasmic bridges (Fig. 156).[1] Plasma-bridges have also been described in the case of embryonic cells of many types and considerable evidence has been produced to show that they may here play an important part in maintaining the unity of the organism.

The facts thus briefly reviewed have led some important modern writers to accept Heitzmann's general conclusion almost in its entirety. A. Meyer,

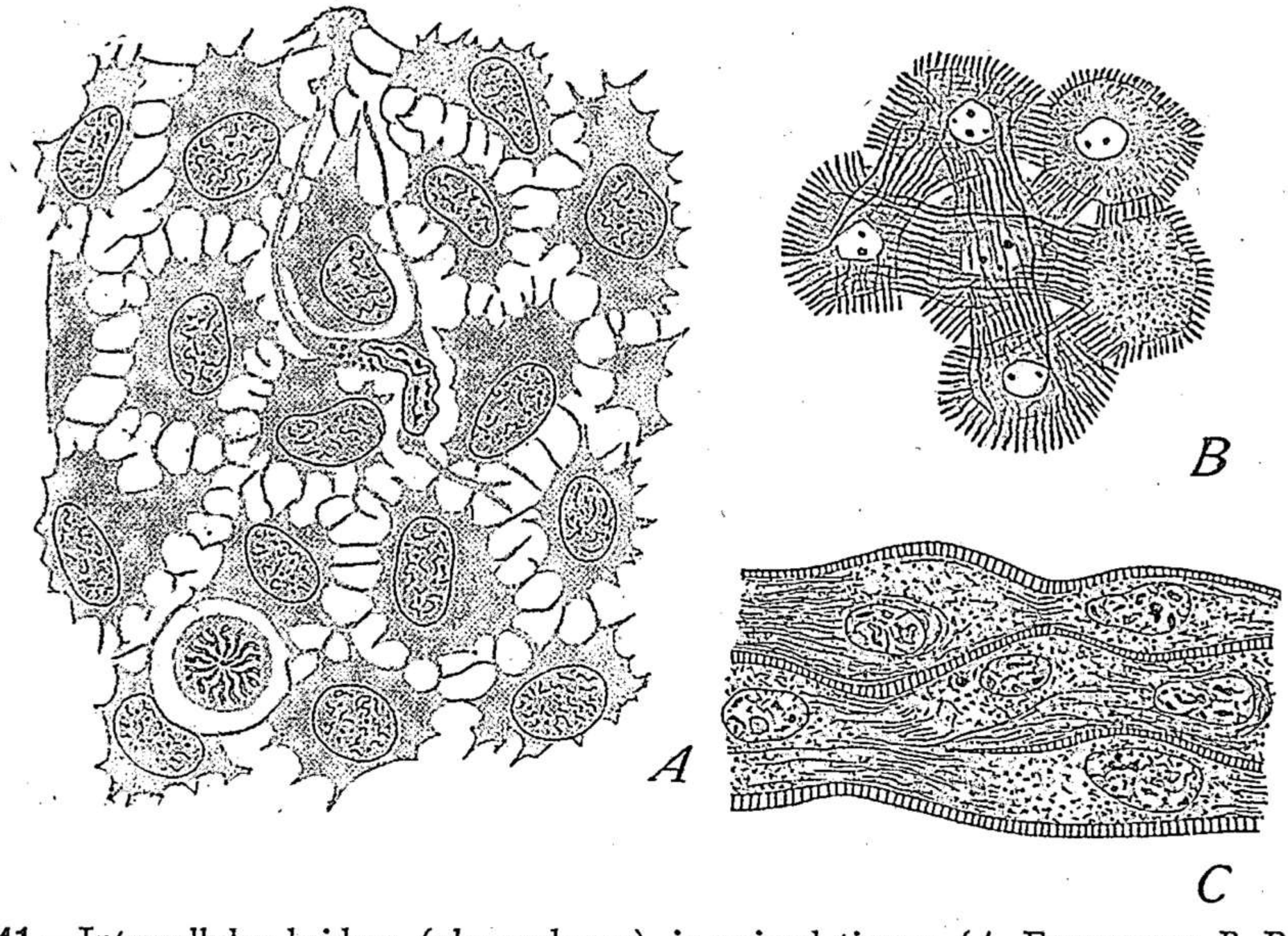

Fig. 41.—Intercellular bridges (plasmodesms), in animal tissues (*A*, FLEMMING; *B*, RIO-HORTEGA; *C*, IDE).

A, epithelium of the gill-lamellæ of salamander-larva, deeper layers in horizontal view; *B*, cells from the mucous membrane of a nasal polypus, fibrillæ traversing the inter-cellular bridges; *C*, human cancer-cells.

for example, expresses the opinion that both the plant and the animal individual is a continuous mass of protoplasm that forms a morphological unit whether it appear in the form of a single cell, a multinucleated cell, or a system of cells.[2] Sachs concluded, more specifically, that "The multicellular plant differs from the unicellular only in that in the one case the protoplasm is traversed by numerous sieve-like or lattice-like plates, while in the other these plates are absent."[3] Adam Sedgwick ('94) endeavored to show that in *Peripatus*, in lower vertebrates, and presumably in animals generally the embryonic cells are in general in direct continuity, the entire body being up to a late stage a continuous syncytium. This conclusion is in harmony with that reached by many experimental

[1] See, for example, Dendy ('88), (sponges), Retzius ('89), (mammals), Goroschankin ('83), (cycads), A. Meyer ('96), (Volvox), Ikeno ('98), (cycads).

[2] '96, p. 212. *Cf.* the views of Hanstein, Strasburger, Russow and others there cited.

[3] Cited from O. Hertwig, '12, p. 491.

embryologists (Wilson, '93, Hammar, '96, '97, etc.) and by some of the ablest students of normal development, such as Rauber ('83), Whitman ('93), and many later writers.

By the earlier botanical observers it was supposed that in the case of plants the plasma-bridges were a consequence of incomplete division and they were even conjectured to be direct derivatives of the spindle-fibers of previous mitoses (Tangl, Russow, and especially Gardiner). Later observers, however, such as Kienitz-Gerloff and Strasburger ('01) opposed this view, so far as the fine fibrils or "plasmodesms" of higher forms are concerned, though admitting that broader connections (as, for instance, in various algæ) may thus arise. The finer bridges are of secondary origin and penetrate the cell-wall secondarily; and it would seem that in some cases the protoplasmic outgrowths from opposite sides of the wall only approach each other closely but without actually uniting. A. Meyer ('96) has shown in *Volvox* that the cell-bridges are formed anew after division; and in like manner Flemming has observed that when the wandering cells or leucocytes creep about among the epithelial cells of the epidermis (of laval salamanders) they rupture the plasma-bridges, which are then formed anew behind them.[1] In harmony with this are the interesting observations of G. F. Andrews ('97) and E. A. Andrews ('98a, b) who have seen the living blastomeres of echinoderm and nemertine eggs spinning numerous delicate protoplasmic filaments which establish secondary connections between the blastomeres subsequent to their separation by division and may even traverse the blastocoele so as to connect widely separated cells.

VII. THE POLARITY AND SYMMETRY OF CELLS

Polarity and symmetry are among the most interesting features of the cell for the student of development; [2] for the polarity of the adult body is modeled on that of the ovum, which in its turn is but a particular case of a phenomenon seen in many other kinds of cells, among both unicellular and multicellular organisms. Fundamentally both the nature and the origin of polarity are unknown (p. 108). We know only its visible expression, which in most cases is both structural and functional, appearing on the one hand in a polarized grouping of the cell-components, on the other in differences of functional or metabolic activity with respect to the axis thus marked off. Which of these (if either) is the more fundamental is an open question, belonging to that ancient and probably barren problem as to

[1] '95, pp. 10–11, '97, p. 261.

[2] The polarity of the animal egg was first made known by von Baer (1834) and further investigated by Remak (1805-55). It was recognized in other forms of cells by Van Beneden ('83, '87) and Rabl ('85, '89).

whether structure or function came first in the order of nature (p. 670). So far as external appearances go it must be said that structural polarity would seem in general to be of secondary origin, of which the egg offers a conspicuous example (p. 1023); but critical consideration of such cases leaves us in doubt as to the underlying aspects of this problem.

Functional polarity in the form of a polarized localization of function is a familiar phenomenon in higher organisms but one that is not easy to investigate apart from the structural dispositions by which it is usually accompanied or preceded. It is strikingly shown in the phenomena of regeneration in plants where, as shown especially by "Vöchting ('85, '92, etc.) even very small pieces (*e. g.*, in *Marchantia*) retain their original polarity, the new apical region being formed typically from or near to the most apical region of the piece; and since these pieces may be very small, Vöchting concluded that every cell is probably polarized in the same sense and may give rise to a complete plant. A similar polarization in relation to regeneration has been observed in various animals, particularly in coelenterates, planarians and annelids, though not in pieces so small;[1] but it has been shown that under certain conditions the direction of polarity may here be experimentally reversed (heteromorphosis). The phemonena of grafting, both in plants and animals, likewise emphasize the physiological polarity of fragments of the organism.

In the case of single cells physiological polarity is seen in the polarized metabolic activities of the germ-cells, gland-cells, many kinds of epithelial cells, the nerve-cells and others in which these activities are more or less clearly expressed by changes, periodic or permanent, in the cell-substance. This was clearly recognized by Remak whose terms "vegetative" and "animal," applied to the poles of the animal ovum, obviously imply a characteristic difference of metabolic activity between them. Child ('11–'16) has recently emphasized the general importance of "metabolic gradients" as an expression (if not the actual cause) of functional polarity, which in his view may sometimes be merely a graded difference in the rate of metabolism in the direction of the axis (though it may often be more than this). In support of this he has proved experimentally, by a study of susceptibility to the action of poisons and narcotics, that such gradients undoubtedly exist in the direction of the main axes, both in organisms as a whole and in individual cells.

Interesting possibilities for the further analysis of physiological polarity are opened by recent experiments. It has been shown that in hydroids the oral region is electronegative as compared with the basal;[2] and also

[1] See especially Morgan ('01), Loeb ('02), Child ('15).
[2] Mathews ('04), Hyman and Bellamy (22), Lund ('23).

that axial differences of electrical potential similar in type, though different in detail, exist in other animals; [1] and Lund has demonstrated that in the alga *Fucus* the polarity of the eggs shows a distinct orientation with respect to the electric field. Hyman and Bellamy (*op. cit.*) emphasize the fact that in the various cases studied by them the electrical gradients closely correspond with the metabolic, levels of high metabolic rate being electronegative to those of lower.

Structural polarity may appear either in the external form of the cell or in a polarized grouping of the various cell-components about an ideal *organic axis* or *cell-axis*. In the cells of higher animals generally, as was first indicated by Van Beneden ('83), the cell-axis is most commonly indicated by the position of the central bodies with reference to the nucleus, the axis passing through the center of both (Fig. 42), while both nucleus and central apparatus are often eccentric towards one or the other pole. This conception was developed by Rabl ('85) who considered that the nucleus likewise shows a polarity corresponding with the cell-axis as thus determined (p. 829). It was carried still further by Heidenhain ('94–'96, etc.) who considered the centers as forming the insertion of persistent astral rays or "organic radii" which extend throughout the cell and by their conditions of tension determine the position and movements of the nucleus and the succession of division-planes in the cell. This particular conception, however, has received little support from later investigation (p. 180).

The cell-polarity as marked out by nucleus and central bodies is often emphasized both by the external form of the cell and by many other of its structural features. The Golgi-bodies, and sometimes also the chondriosomes, are often grouped about the centers or oriented with respect to them (p. 50). In epithelial cells generally the centers, usually double in the form of a "diplosome," typically lie towards the free surface, often almost at the periphery, thus marking an axis that is vertical to the surface (Fig. 42). In the direction of this axis the cell is often elongated (as in the columnar epithelia), and the basal and peripheral regions of the cytosome as thus marked often show conspicuous differences of metabolic activity accompanied by corresponding morphological differentiations. This is shown with especial clearness in many forms of gland-cells (pancreas, salivary glands), in columnar ciliated cells, and above all in the germ-cells, in all of which the structural polarity is often manifested by a conspicuous stratification or polarized grouping of formed elements such as granules, yolk-spheres, pigment and the like. This grouping does not, however, in itself constitute the basis of polarity, as has been demonstrated by centrifuging eggs and

[1] In ctenophores the electronegativity is greatest at the aboral pole; in platodes and annelids both ends are electronegative to the middle. (*Cf.* Morgan and Diman '04.)

other cells by which these bodies may be caused to undergo marked dislocation without displacing the axis itself, as is shown by the later history of the cell (p. 1089).[1] Both nucleus and centers likewise, may be displaced, either by centrifuging or by mechanical pressure; and both may move extensively through the cytoplasm under normal conditions, without changing the cell-polarity, as we see, for example, during the fertilization and cleavage of the egg (p. 425). Polarity, finally, appears in many forms of plant-cells in which central bodies are absent or are represented by much

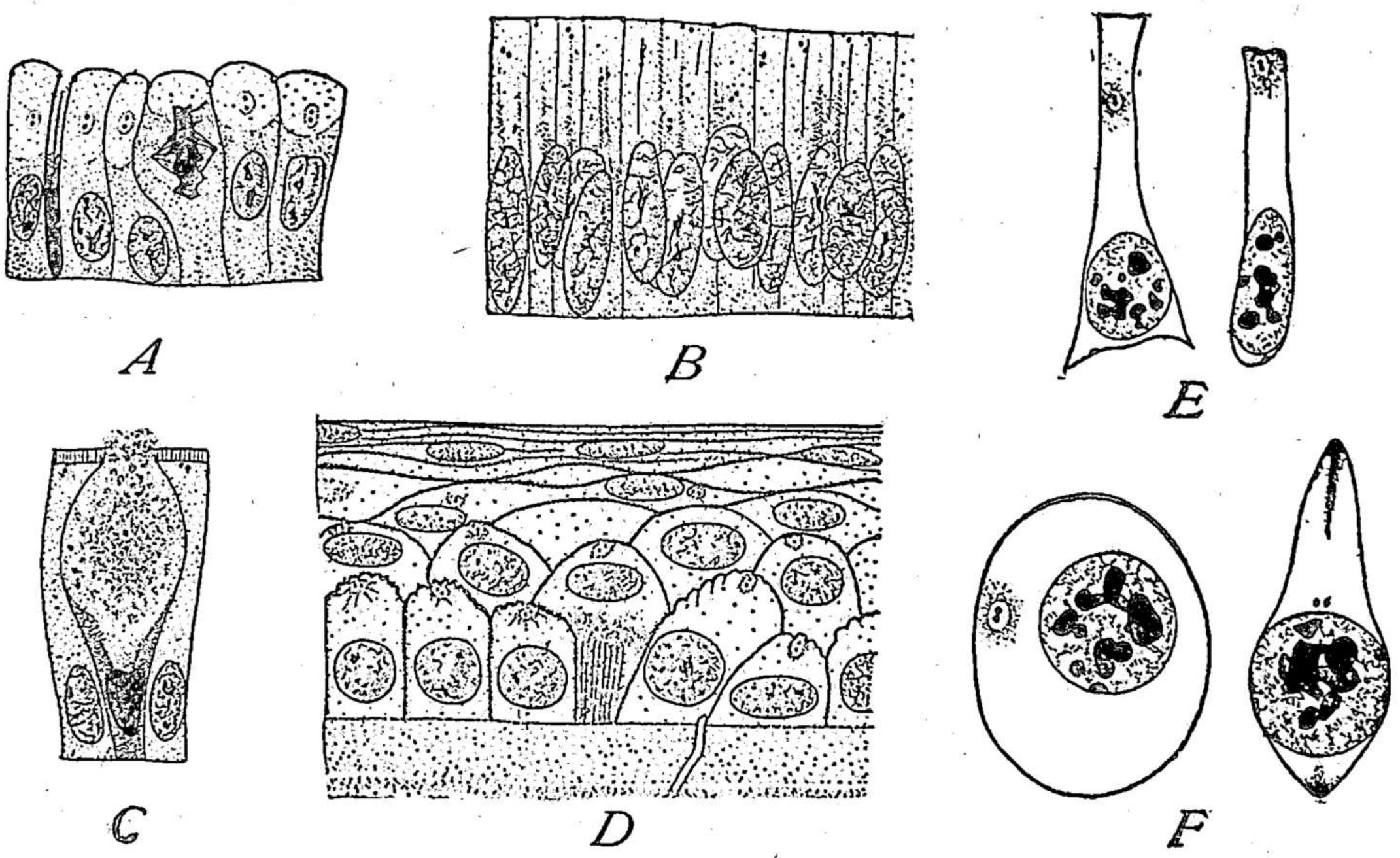

Fig. 42.—Central bodies (centrioles), in epithelial and other cells (*A–D*, ZIMMERMANN; *E*, HEIDENHAIN and COHN; F, HEIDENHAIN).

A, from gastric glands of man; dead cell at the left. *B*, uterine epithelium, man; *C*, from human duodenum; goblet-cell, with centriole in the middle; *D*, corneal epithelium of monkey; *E*, epithelial cells from mesoblast-somites, embryo duck; *F*, red blood-corpuscles from the duck-embryo. The centrioles are double in nearly all cases.

larger and less clearly defined structures, *e. g.*, in the somatic cells of *Isoëtes* or in the synaptic stages of the sporocytes of *Marsilia* and *Equisetum* (Marquette, '07).

All this has led many observers to the conclusion that the fundamental basis of polarity must be sought in the continuous and apparently homogeneous hyaloplasm of the egg ("ground-substance" of Lillie, "spongioplasm" of Conklin).[2] In harmony with this is the fact that in many ova

[1] Gurwitsch ('08), Lyon ('07), Lillie ('08, '09), Morgan ('08, '00), Boveri ('10), Morgan and Spooner ('00), Conklin ('10, '12, '16, '17) and others.

[2] "Polarity is not a result of the position of the nucleus or of any configuration of granules. It must depend upon some configuration or heterogeneous physical or chemical properties of the ground-substance established early in the history of the egg, and which is not essentially disturbed by centrifuging" (Lillie, '09).

the polarized grouping of pigment, yolk and the like is visibly attained by a secondary process of segregation, often not effected until near the time of maturation and fertilization, but conforming to a preëxisting axis marked by the point of attachment of the egg, the position of the micropyle, the eccentricity of the nucleus, or by other characters (p. 1094). It is also in harmony with the earlier conclusion of Driesch ('96, '98), based on displacement of the nuclei and centers by mechanical pressure and on the development of egg-fragments, that the position of the nuclei and centers is non-essential, and that polarity and bilaterality belong to the protoplasmic substance as such (hyaloplasm) irrespective of the formed elements that it may contain (p. 1019).[1]

Interesting questions are thus raised concerning the organization of the hyaloplasm. Both Driesch and Boveri argued in favor of a "polar-bilateral orientation" of the ultimate protoplasmic particles that make up the "intimate structure" of the egg. Lillie and Conklin alike concluded, further, that the hyaloplasm is relatively solid, *i. e.*, in high degree viscous. Lillie at first ('06) held the view, based on the Brownian movements of the microsomes (p. 61), that the hyaloplasm is a "fluid medium," but later ('09) concluded that it is "finely organized" and that the flowing movements that it seems to perform are an illusion produced by movements of the granules through it. Conklin considers the hyaloplasm as forming a framework of "spongioplasm" traversing a more fluid substance, in which the granules, etc., are suspended, and through the elasticity and contractility of which are determined the positions of all the included structures (nucleus, central bodies, granules, etc.) and their return to their normal positions after artificial displacement.[2]

Accepting the general conclusions thus indicated we can readily understand how the various inclusions and other intracellular structures may be shifted about without changing the direction of the cell-axis. We may also see how the cell-axis itself, persistent as it is when once established, may originally be laid down in this direction or that by an epigenetic process; and here, probably, we find the most reasonable interpretation of the fact that the direction of the axis is so often correlated with the relation of the cell to its immediate environment (as in columnar epithelial cells or the ovarian egg).

Van Beneden expressed the opinion that bilateral symmetry is likewise a widespread if not universal phenomenon among cells, at least in bilateral animals. This, however, has received little support from later researches.

[1] On this point see especially Boveri, '01.

[2] An interesting light is thrown upon these results by the work of Heilbrunn and Chambers on the changes of viscosity during mitosis (pp. 197, 1092).

Among Protista, it is true, there are certain forms (ciliates, flagellates and some of the unicellular plants) that are more or less distinctly bilateral; in some cases showing differentiated axes which have the same general relation to the environment and the movements of the individual as in higher forms (Fig. 43). It is also true that the eggs of insects and cephalo-

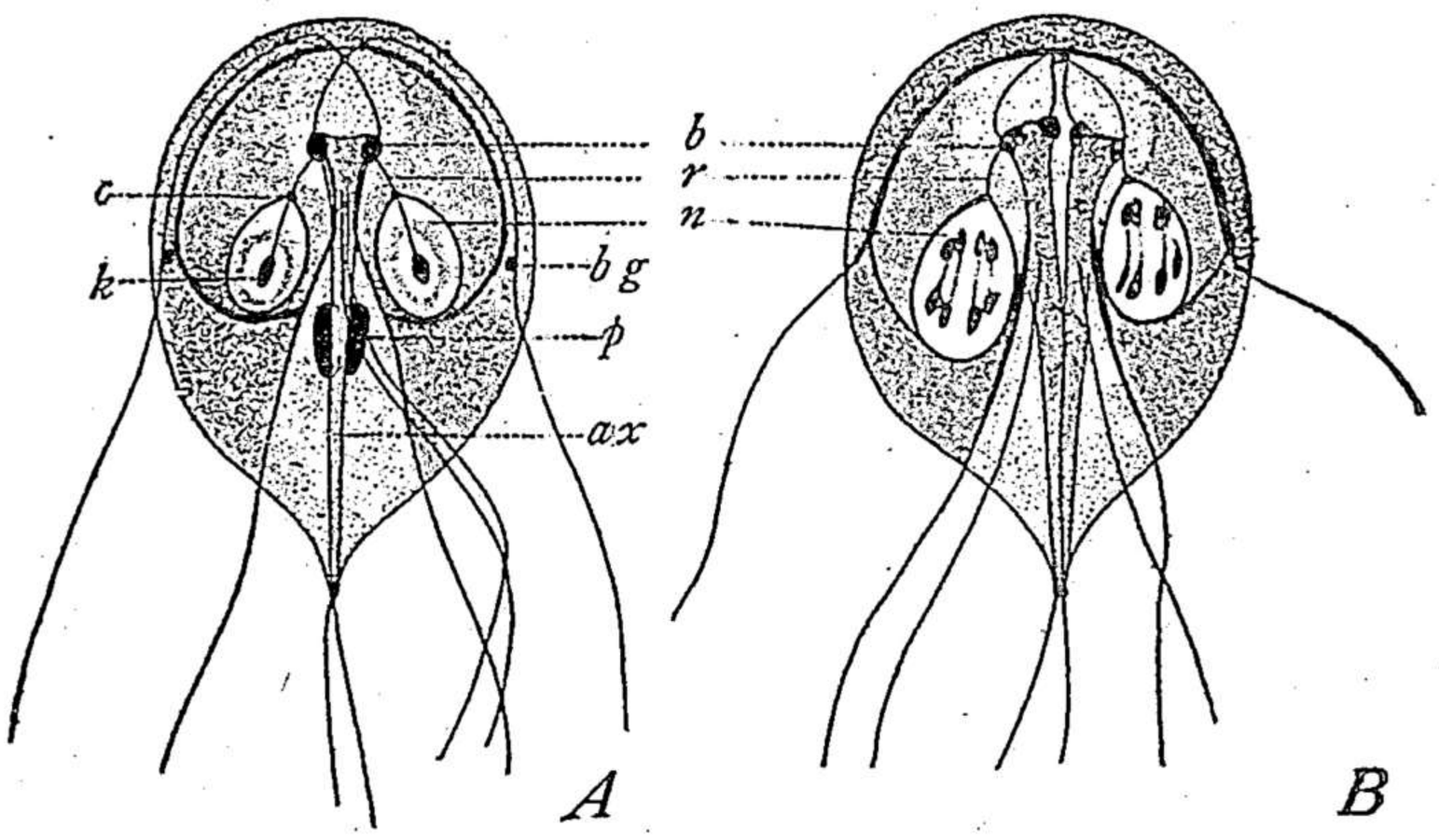

Fig. 43.—A bilateral, binucleate flagellate, *Giardia muris*, showing flagella and basal apparatus (KOFOID and CHRISTIANSEN).

A, in the ordinary vegetative state, *B* in mitosis, the axostyle and blepharoplasts double.

ax, axostyle; *b*, blepharoplasts; *bg*, basal granules; *k*, karyosome; *n*, nucleus; *p*, parabasal body; *r*, rhizoplast.

pods and the sperms of some species of animals are bilateral, both in form and in structure (pp. 276, 374). A certain amount of support for considering the cells of columnar epithelium as bilateral structures was found by Heidenhain ('99). Nevertheless it must be said that there is little ground for regarding bilaterality as characteristic of cells generally; and as applied to the somatic cells Van Beneden's conclusion wears a somewhat transcendental aspect.

LITERATURE I

(See also VIII, XII, etc. For abbreviations see p. 1145).

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CHAPTER II

CELL-DIVISION

"Where a cell exists there must have been a preëxisting cell, just as the animal arises only from an animal and the plant only from a plant. The principle is thus established, even though the strict proof has not yet been produced for every detail, that throughout the whole series of living forms, whether entire animal or plant organisms, or their component parts, there rules an eternal law of *continuous development*."

VIRCHOW.[1]

It is now sixty years since Virchow first adequately stated the principle of genetic continuity of cells by division, which was destined to form the rallying point for all future conceptions of heredity and development. Only a minute fraction of the vast field of cytology and embryology had then been examined, and Virchow's celebrated aphorism *omnis cellula e cellula* [2] was too far in advance of his time to appear in its true proportions. As years passed, it gradually became evident that this terse phrase embodies one of the most important generalizations of modern science. The advance of cytological research still continues day by day to add fresh weight to the demonstration that cells have no other mode of origin than by the division of preëxisting cells. [3] In this respect a fundamental likeness exists between unicellular organisms, the tissue-cells of higher plants and animals, and the germ-cells from which the higher organisms take their origin. Upon cell-division, therefore, depends not alone heredity but the very continuity of life.

The division of cells was probably first seen in the segmentation of the animal egg (Prévost and Dumas, 1824), and soon afterwards in the lower plants by several botanists; [4] but its significance was not fully recognized until after the promulgation of the cell-theory, as a result especially of the work of Kölliker, Remak and Virchow. During the first two decades following Schleiden and Schwann these observers, together with the botanists Mohl, Nägeli and others, were accumulating the proof that cells arise only by the division of preëxisting cells and that the authors of the cell-theory fell into error when they accepted the independent origin of cells

[1] *Cellularpathologie*, p. 25, 1858.

[2] *Cf.* Introduction, p. 11.

[3] Division may be equal (fission), unequal (gemmation or budding) or endogenous (usually multiple).

[4] Brogniart, Meyen, Mirbel, Mohl, 1827–1835.